VII\textsuperscript{th} International Congress of Andrology

June 15-19, 2001
Montréal, Canada

Program and Abstracts
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstracts</td>
<td>82</td>
</tr>
<tr>
<td>Author Index</td>
<td>205</td>
</tr>
<tr>
<td><strong>Congress Program</strong></td>
<td></td>
</tr>
<tr>
<td>Friday, June 15</td>
<td>30</td>
</tr>
<tr>
<td>Saturday, June 16</td>
<td>31</td>
</tr>
<tr>
<td>Sunday, June 17</td>
<td>34</td>
</tr>
<tr>
<td>Monday, June 18</td>
<td>38</td>
</tr>
<tr>
<td>Tuesday, June 19</td>
<td>40</td>
</tr>
<tr>
<td><strong>Course Objectives</strong></td>
<td></td>
</tr>
<tr>
<td>and CME Credit Information</td>
<td>26</td>
</tr>
<tr>
<td>Distinguished Andrologist Award</td>
<td>23</td>
</tr>
<tr>
<td>Distinguished Service Award</td>
<td>24</td>
</tr>
<tr>
<td>General Information</td>
<td>18</td>
</tr>
<tr>
<td>Information for non-Canadian Colleagues</td>
<td>20</td>
</tr>
<tr>
<td>Laboratory Science Forum</td>
<td>19</td>
</tr>
<tr>
<td>List of Exhibitors</td>
<td>12</td>
</tr>
<tr>
<td>List of Sponsors</td>
<td>8</td>
</tr>
<tr>
<td>Local Organizing Committee</td>
<td>9</td>
</tr>
<tr>
<td>Chair’s Message</td>
<td>9</td>
</tr>
<tr>
<td>New Investigator Award</td>
<td>26</td>
</tr>
<tr>
<td>Past Presidents of ASA</td>
<td>8</td>
</tr>
<tr>
<td>Past Presidents of ISA</td>
<td>2</td>
</tr>
<tr>
<td>President’s Poster Session (List of Abstracts)</td>
<td>186</td>
</tr>
<tr>
<td>Press Relations and News Coverage</td>
<td>12</td>
</tr>
<tr>
<td>Poster Session 1/2 (List of Abstracts)</td>
<td>44</td>
</tr>
<tr>
<td>Poster Session 3/4 (List of Abstracts)</td>
<td>55</td>
</tr>
<tr>
<td>Poster Session 5/6 (List of Abstracts)</td>
<td>66</td>
</tr>
<tr>
<td>Postgraduate Course I</td>
<td>29</td>
</tr>
<tr>
<td>Postgraduate Course II</td>
<td>36</td>
</tr>
<tr>
<td>President’s Welcome Message (ASA)</td>
<td>5</td>
</tr>
<tr>
<td>President’s Welcome Message (ISA)</td>
<td>3</td>
</tr>
<tr>
<td>Program Organizing Committee</td>
<td></td>
</tr>
<tr>
<td>Chair’s Message</td>
<td>9</td>
</tr>
<tr>
<td>Registration Information</td>
<td>18</td>
</tr>
<tr>
<td>Serono Award Lectureship</td>
<td>22</td>
</tr>
<tr>
<td>Society Leadership (ASA)</td>
<td>4</td>
</tr>
<tr>
<td>Society Leadership (ISA)</td>
<td>2</td>
</tr>
<tr>
<td>Travel Information</td>
<td>21</td>
</tr>
<tr>
<td>Women in Andrology</td>
<td>19</td>
</tr>
<tr>
<td>Young Andrologist Award</td>
<td>25</td>
</tr>
</tbody>
</table>

## Notice to Readers

Every effort has been made to ensure that the information printed here is correct; however, details are subject to change. Be sure to check the reader board for the most up-to-date information while you are at the meeting.
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www.isa2001.org
Welcome from the ISA President

Dear Andrologist,

It gives me great pleasure, on behalf of the International Society of Andrology (ISA), to welcome everyone to the VIIth International Congress of Andrology (ICA) in Montréal, Canada. The Local Organizing Committee headed by Dr. Carlos R. Morales, has done a superb job with the local arrangements, aiming to provide the best possible conditions for this important Congress. The scientific program has been compiled by the Program Organizing Committee (POC) headed by Dr. Héctor Chemes (Buenos Aires, Argentina). The members of the POC represent all the major disciplines of andrology, as well as the breadth of regions our Society encompasses. The program reflects the most recent developments of all the increasingly diverse fields that comprise modern andrology. At the same time, the program has been designed to balance the interests of those working in the clinical and basic science aspects of andrology. Finally, special care has been devoted to the social program, which will make all of our stay an unforgettable cultural experience. We trust you will both enjoy and be enriched by participating fully in the Congress.

The International Congress of Andrology, held every four years, is the major and most visible activity of the ISA. It has become a tradition that these Congresses circulate between the different continents. The first ICA was held in the year 1976 in Barcelona, the second Congress in 1981 in Tel Aviv. The 3rd Congress was the last one in America, in Boston 1986. The 4th, 5th and 6th Congresses were 4 years apart in Florence (Italy), Tokyo (Japan) and in Salzburg (Austria) and now we convene for the 7th ICA in Canada. The venue of the 8th ICA will be voted for by the ISA General Assembly Meeting during this Congress.

ISA has today grown from a small association of a handful of visionaries to a full-size international scientific society, with over 8000 members in a total of 38 national and regional member Societies, covering each continent. In addition to organizing the International Congress of Andrology, the other main activities of ISA are to provide expertise and leadership in basic and clinical issues of andrology, participate in organization of regional and special meetings and workshops in the field of andrology, and above all, promote scientific andrology world-wide. Fostering knowledge of andrology in less developed areas of the world has always been a particularly strong interest of ISA. In these leadership activities, ISA has long had a partnership with the World Health Organization. In recognition of this, ISA has recently been granted formal status of a Non-Governmental Collaborating Organization with WHO. It has always been one of ISA’s priorities to provide funding for young scientists from developing countries to attend the ISA Congresses.

Andrology is a relatively new field among medical and scientific specialties. While it originated from the concern to embody the highest levels of scientific expertise in clinical and laboratory evaluation of infertile men, andrology has now grown to encompass all aspects of male reproductive health, and even more widely to non-reproductive aspects of men’s health. The main challenges of andrology today are male infertility, male contraception, sexually transmitted diseases and health issues of the ageing male including prostate disease and the role of hormone replacement. All of these topics will be addressed at the Congress by experts in andrology and it’s allied fields. We trust that the Congress will fulfill, both scientifically and socially, the expectations of all participants. And therefore, on behalf of the ISA, I am proud to welcome you all to Montréal.

Ilpo T. Huhtaniemi, M.D., Ph.D.
President, International Society of Andrology
American Society of Andrology

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2001 VIIth ICA Postgraduate Course:
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2001 VIIth ICA Program:
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2002 Program:
2003 Local Arrangements:

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Constitution & Bylaws:
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Finance:
Future Meetings:
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Membership:
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Welcome from the ASA President

Dear Colleague:

Welcome to Montréal! The American Society of Andrology (ASA) considers it a great honor to be the host Society for the VIIth International Congress of Andrology. Montréal is a lovely vibrant city and the Congress agenda, both scientifically and socially, is outstanding. Everyone attending the Congress is sure to find this a most rewarding experience.

The success of the Congress is owed to many who have spent literally years of effort in planning and preparation. Dr. Carlos Morales, Chair of Local Organizing Committee, and his Committee members have diligently worked to assure that the meeting runs smoothly, that physical arrangements are superb, and that the social events are enjoyable occasions where new and old acquaintances can be fostered.

In parallel, the International Program Organizing Committee, chaired by Dr. Héctor Chemes, has put together an incredible scientific program of internationally renowned speakers who will deliver state-of-the-art information on a broad spectrum of topics. These days, having an interest in "andrology" encompasses many disciplines, including molecular, and clinical endocrinology and neuroendocrinology, physiology, developmental biology, gamete and cell biology, toxicology, aging, contraception and infertility, prostate, sexual function, and ethics. This Congress program has something for everyone! In addition to the regular Congress program, there are two postgraduate courses being offered; one course, chaired by Dr. Jonathan Jarow and Dr. Serge Carrier, will cover recent advances in clinical andrology, while the second course, chaired by Dr. Christopher De Jonge and Dr. David Mortimer, will offer practical information for the andrology laboratory.

Many other people have been involved with making this Congress a reality, but I especially want to thank Sabrina Ritchie and Cherokee Melton of the ASA Executive Office, and Lucy Felicissimo & Associates, Inc., PCO in Montréal, for all their hard work. Organizing, coordinating, and tending to all the details that make a meeting such as this a success requires special skills, conscientiousness, and a gift for patiently and pleasantly working with scientists and physicians.

It also would be difficult to produce a Congress such as this without the generous and much appreciated support of industry and other organizations. Through their sponsored lectures, programs and exhibition booths, attendees have access to the latest in knowledge, clinical and scientific products, and technological advances. We are very grateful.

For those who are ASA members, I want to personally thank you for the privilege of being your President for this past year. There have been a number of difficult issues to tackle and some hard decisions to make, but with the terrific support and guidance of the Society Council, your officers (Vice-President Dr. Barry Zirkin, Secretary Dr. Dolores Lamb, and Treasurer Dr. Christopher De Jonge), and the Society Committee Chairs, especially Dr. Gail Prins, I believe the Society is on track to continue in its important role of "advancing and promoting knowledge of the male reproductive tract and Andrology". The ASA may not be large a Society, but it is unique and very special. May it continue to flourish!

J. Lisa Tenover, M.D., Ph.D.
President, American Society of Andrology
Floorplan – Montréal Convention Centre – 4th floor

Montréal Map

Congress Venue & surroundings
### Agenda “at a glance”

<table>
<thead>
<tr>
<th>Time</th>
<th>FRIDAY</th>
<th>SATURDAY</th>
<th>SUNDAY</th>
<th>MONDAY</th>
<th>TUESDAY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June 15</td>
<td>June 16</td>
<td>June 17</td>
<td>June 18</td>
<td>June 19</td>
</tr>
<tr>
<td>08:00</td>
<td>Plenary Lecture 2</td>
<td>Plenary Lecture 4</td>
<td>Plenary Lecture 6</td>
<td>President’s Poster Presentations</td>
<td></td>
</tr>
<tr>
<td>09:00</td>
<td>Symposium 1-3</td>
<td>Symposium 7-9</td>
<td>Symposium 12-14</td>
<td>Health Break (9:30-10:00)</td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>Health Break</td>
<td>Health Break</td>
<td>Health Break</td>
<td>Symposium 15-17 (10:00-11:30)</td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>Poster Session 1</td>
<td>Poster Session 3</td>
<td>Poster Session 5</td>
<td>ASA Awards (11:30-11:45)</td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td>Workshops 1-3</td>
<td>Workshops 4-6</td>
<td>Workshops 7-9</td>
<td>AUA Plenary Debate (11:45-12:45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lunch Break</td>
<td>Lunch Break</td>
<td>Women in Andrology Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lab Science Lunch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td>Poster Session 2</td>
<td>Poster Session 4</td>
<td>Poster Session 6</td>
<td>Conclusion (12:45)</td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td>Health Break</td>
<td>Health Break</td>
<td>Health Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td>Symposium 4-6</td>
<td>Symposium 10-11</td>
<td>ISA Business &amp; General Assembly</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Debate</td>
<td></td>
<td>ISA Travel Awards</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td>Plenary Lecture 3</td>
<td>Plenary Lecture 5</td>
<td>ASA Annual Business Meeting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td>Opening Ceremony</td>
<td>Award</td>
<td>Awards</td>
<td>Banquet <em>(Buses depart at 18:15)</em></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td>Plenary 1</td>
<td>Student Mixer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td>Opening Mixer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Years</th>
<th>President</th>
<th>Years</th>
<th>President</th>
</tr>
</thead>
</table>

* Deceased
Welcome from the Local Organizing Committee

Dear Colleague,

On behalf of the Local Organizing Committee, I extend a warm welcome to the participants, accompanying members, exhibitors and sponsors to the VIIth International Congress of Andrology. This Congress promises to be one of the most memorable events in the scientific calendar for 2001.

Andrology is an extensive discipline encompassing reproductive medicine, endocrinology, urology, and various aspects of cellular and molecular biology. Being a relatively new field, andrology faces many exciting challenges in different aspects of male reproductive health, such as male infertility, contraception, sexual dysfunction, sexually transmitted diseases, toxicology, androgen action, ageing and cancer. A number of new developments and approaches are also attracting the attention of andrologists, such as the use of stem cells, gene transfer and germ cell engineering. Some other aspects of andrology are closely linked to advances in reproductive technology (cloning, ART/ICSI, etc.) and our discipline possesses all the tools to provide answers to controversial scientific and ethical issues.

The scientific program has been arranged by an international Program Organizing Committee to feature leaders in basic and clinical andrology, from specialized topics to general areas of clinical importance. This World Congress has gathered companies in the business of pharmaceuticals, diagnostics, medical and scientific equipment, biotechnology research and development, publishers of medical and scientific books, journals and electronic media, whose products are relevant to reproductive health and reproductive technology.

Due to the size of the meeting (around 1000 participants) the International Congress of Andrology is being held at the Montréal Convention Center (Palais des Congrès de Montréal). The Convention Center is located in downtown Montréal, near Chinatown and Old Montréal, and at a walking distance from the two official Congress hotels. In addition, the participants have easy access to the subway system from a station located under the Convention Center. The Palais offers a modern, high-tech conference setting with the most advanced communication systems available.

To make this occasion more enjoyable, we have chosen Montréal in the middle of June as the Congress venue, because of the multiple cultural activities that take place during the early summer in our city. This month of the year is also the most beautiful time in Montréal. Thus, along with the multidisciplinary scientific program, many socio-cultural activities can be planned. Tourist and general information will be available in the registration area. Some special events are anticipated before and during the week of the meeting, such as the Formula 1 Grand Prix of Canada, the International Fire-Work Competition, and the performance of the week of the Montréal Symphonic Orchestra (within walking distance of the official Congress hotels). The Museum of Architecture, the Museum of Fine Arts of Montréal, the McCord Museum of McGill University and the Botanical Garden, featuring an exhibit of Bonsai trees, will also be open (all accessible via a short Metro ride). Finally, you can also enjoy "Old Montréal" and the "Old Port" at night. You can walk along Ste-Catherine Street, the bustling "main street" of the city, or along St-Laurent Street, where you can find Greek, Portuguese, German and Italian restaurants.

We consider your participation and support most important to the success of the VIIth International Congress of Andrology and to the realization of its goals. We are looking forward to providing you with a profitable Congress and an unforgettable cultural experience.

Carlos R. Morales, D.V.M., Ph.D.
Chair, Local Organizing Committee
Welcome from the Program Chair

Dear Colleagues,

It is a wonderful opportunity to introduce you to the scientific program developed by the Program Organizing Committee for the VIIth International Congress of Andrology in Montréal. We are already beyond the Y2K and this reminds me, as it probably does for many of you, the many expectations we projected for the future, which is now our present. If you find, as we hope you will, that this program synthesizes the current developments and anticipates future trends, we will feel happy to have accomplished the goal of fostering the progress of andrology as a crossroads for basic biological research and as a scientifically based medical discipline.

The program is organized into 7 Plenary Sessions, 18 Symposia, 9 Workshops and 2 Postgraduate Courses. We have been fortunate to gather a group of outstanding scientists who are internationally recognized as leaders in their fields of expertise and are also excellent speakers.

Our keynote speaker is Dr. John Gearhart who has developed pioneering work on human stem cells derived from primordial germ cells. His Serono Lecture will be on Stem Cells, Germ Cell Engineering and Cloning. The work on the biology of human stem cells has been highlighted by Science as the breakthrough of the year in 1999. This lecture will be echoed in the Symposium on Germ Cell Transplantation, in vitro Development and Transgenesis in which the potential for stem cells to repopulate the infertile testis and the prospects for genetic modification will be explored. The role of stem cells and primordial germ cells in the biology, epidemiology and treatment of Testicular Cancer will be explored in another symposium.

The roles of novel genes in spermatogenesis will be the topic of the plenary lecture delivered by Dr. Mary Ann Handel. Recent developments highlighting possible genetic involvement in human infertility will be dealt with in the symposia on Animal Models of Male Infertility and FSH in the Male, in which the controversial role of FSH in human spermatogenesis will be revisited.

Dr. Albert Brinkman, the Ernst Schering Research Foundation lecturer, will address recent progress on different aspects of androgen action in health and disease. The role of androgens and other steroids in biological and behavioral aspects of CNS function and as contraceptive agents will be addressed in Androgens and Brain and in Male Contraception.

Dr. Wayne Hellstrom will present current concepts on Advances in Male Sexual Dysfunction, a topic which will be further explored in a symposium in which the physiopathology of MSD as well as medical and surgical approaches to therapy will be discussed, including Peyronie’s disease.

The innovative therapies that have revolutionized the treatment of severe male factor infertility will be discussed by Dr. Herman Tournaye, in Treating Male Infertility by ART-ICSI. Medical and ethical concerns that surfaced with the introduction of these novel methods will be the subject of an exciting debate in Ethics in Andrology moderated by Dr. Michael McClure.

We have asked Dr. John McKinlay to review the increasingly important subject of the hypogonadal state and androgen deficiency in ageing males, discussing recent developments of the Massachusetts Male Ageing Study. The symposium on Androgen therapy and Ageing will focus on specific aspects of the effects of androgens in older men.

An interesting feature of this program is the introduction of debates in topics of wide interest like that of Ethics in Andrology, as previously discussed. Dr. Fernand Labrie and Dr. Michael Marberger, in the American Urological Association Plenary Debate moderated by Dr. Claude Schulman, will expose diverging views on screening for Prostate Cancer. Novel aspects of prostate visualization as well as hormonal and chemotherapeutic therapies will be dealt with in the symposium on Prostate Disease.
The Symposium on Cell Survival and Death will focus on the general mechanisms that regulate the cell cycle and programmed cell death, in particular those operative in spermatogenesis and in the prostate. The cell and molecular biology of the Epididymis, including the role of epididymal proteins in fertilization and the mechanisms of Sperm-Egg recognition and interaction will be explored in two symposia.

The role of specific proteins in the synaptonemal complex, the cytoskeleton of the sperm head and novel components of the sperm fibrous sheath will be described in Sperm Structure and Function.

The significance of Inhibin and Anti-Müllerian Hormone as recently explored indicators of testicular and prostate function will be the topic of Non Traditional Markers of Reproductive Tract Function, and the influence of genetic factors in the diversity of responses of different populations will be presented in Clinical Genetics and Population Variation in Andrology.

The need for well conducted epidemiological studies in assessing population trends and the enormous impact of the rapidly expanding HIV pandemic and other infections that are sexually transmitted will be covered in Public Health Epidemiology aspects of Andrology and in Sexually Transmitted Diseases.

The 9 workshops will concentrate on translating the meaning of new methodologies and their impact in the advance of modern Andrology. The topics will cover areas of clinical interest such as sperm retrieval, the impact of genetic studies in the infertile male and diagnosis of intersexual states. Two workshops will concentrate in methods that originated in basic science laboratories that will surely have an impact in our understanding of male infertility: Germ Cell Transplantation and Transgenic Technology. New concepts on Hormone Assays, Drug Discovery and Design, Proteomics of the cell Map and DNA Chips will contribute the developments of state of the art science to the promotion of research and medical practice.

The program will be preceded by a Postgraduate Course on Recent Advances in Clinical Andrology chaired by Dr. Jonathan Jarow and Dr. Serge Carrier, and will be followed by another Postgraduate Course on the Andrology Laboratory chaired by Dr. Christopher De Jonge and Dr. David Mortimer. Various topics will be presented by excellent speakers who will share their expertise in the areas of wide interest.

This VIIth International Congress of Andrology will also draw its potential from the numerous free communications, in the form of posters contributed by members, guests and trainees. This is a sure opportunity for scientific interaction and collaboration.

The program just outlined is the result of the hard work of the POC that was fortunate to benefit from the generous feedback from numerous colleagues who contributed their input and from the enthusiastic collaboration of the more than 80 invited speakers and session chairpersons.

Let’s make this a memorable occasion for a fruitful exchange of ideas. Thank you all for your support.

Héctor E. Chemes, M.D., Ph.D.
Program Committee, Chair

Program Organizing Committee:
Daniel R. Franken (South Africa)
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Contact:  Mr. James Murphy

**Exhibitors**

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Contact: Ms. Shaunna Salzetti

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E-mail: info@conrad.org  
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General Information

Congress Site
The VIIth International Congress of Andrology will be held at the Palais des Congrès (Montréal Convention Center), located at: 201 Viger Avenue West, Montréal. The Convention Center is located downtown near Chinatown and Old Montréal. Participants will have easy access to the subway system from a station located under the Convention Center.

Registration
Registration forms must be submitted with a check, money order, or appropriate credit card information and returned to the Congress Office (74 New Montgomery, Suite 230, San Francisco, CA 94105, USA). All checks must be in US dollars and drawn on a US bank. All registrations received after April 20, 2001 will be assessed a $75 late fee (including on-site registration). Official registration forms can be downloaded from the Congress website (www.isa2001.org) and online registration is available from the ASA website www.andrologysociety.com. Cancellations must be made in writing and are subject to a $100 cancellation fee prior to April 20, 2001. After that date, no refunds will be given.

For additional information on the VIIth International Congress of Andrology please contact the Congress Office at 74 New Montgomery, Suite 230, San Francisco, CA 94105, USA. You may contact the Congress staff by phone (415-764-4823), by fax (925-472-5901), or by email (asa@hp-assoc.com). We also invite you to visit the following websites: www.andrologysociety.com and www.isa2001.org

The meeting registration and information desk will be located on the fourth floor of the Convention Center, next to the escalators. The desk will be open at the following times for on-site registration:

<table>
<thead>
<tr>
<th>Date</th>
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<tr>
<td>Friday, June 15, 2001</td>
<td>13:00 - 18:00</td>
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<tr>
<td>Saturday, June 16, 2001</td>
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<td>Sunday, June 17, 2001</td>
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<td>Monday, June 18, 2001</td>
<td>7:00 - 18:00</td>
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<tr>
<td>Tuesday, June 19, 2001</td>
<td>7:00 - 12:00</td>
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Registration for the both Postgraduate Course I and II will be at the Wyndham Hotel, Convention Level.
The registration and information desk will be open:
Thursday, June 14 (Alfred Rouleau Room) 7:00 – 17:00
Wednesday, June 20 (Grand Salon B) 7:00 – 15:30

Social and Accompanying Persons Program
Congress delegates and registered accompanying persons are invited to attend the Opening Ceremony, to be held Friday, from 19:30-20:30. The Official Banquet will take place on the Cavalier Maxim, a newly refurbished river boat, and the cruise will be from 19:00-22:30. Motorcoaches will depart from the two Congress hotels (The Inter-Continental and Wyndham) at 18:15 and travel to the Pier Tower. Motorcoaches will shuttle delegates and guests back to the Inter-Continental and Wyndham Hotels at 22:30, after the cruise. Accompanying members will receive a complimentary personalized ticket for this event. Tickets will be sold on a first come/first serve basis.

On Saturday, June 16, accompanying persons will have access to a free tour of Montréal (9:15 - 12:00) following a continental breakfast and an orientation lecture, held in the Convention Center, Room 410 C. This same room will be made available throughout the Congress as a guest lounge for accompanying members.

Press Relations and News Coverage
Dr. Robert Sullivan will coordinate news coverage and encourage publicity of the Congress. He will work with both Congress participants and the news media to bring cutting-edge research before the public eye. A Press Room will be provided in the Convention Center and we hope that delegates, if requested, are willing to discuss their work with the media.

Student Mixer
The student mixer will be Saturday, June 16, from 18:30 – 19:30, in the Convention Center, Room 311. All student and trainee delegates are encouraged to attend. Refreshments and hors d’oeuvres will be served.
Women in Andrology Luncheon

The Women in Andrology group will hold its annual business meeting on Monday, June 18 at 12:30 in the Convention Center, Room 410B. The meeting will include a presentation by Catherine Jay Didion, of the Association for Women in Science, who will give a talk entitled, "Current Issues Facing Woman in Science". Catherine Jay Didion will discuss the current climate for women in science, including: the transition between education and professional work, career development, and combining a successful career with family life. Didion will also discuss ways to encourage women to study the sciences and to pursue a science career, including mentoring in the sciences, and networking students with the professional world. Lunch tickets are available for $25USD and may be purchased by contacting the Congress Office. You may also buy tickets on-site at the main registration and information desk.

Laboratory Science Forum

The 2001 Laboratory Science Forum Lunch will be held Sunday, June 17, from 12:30-13:30, at the Convention Center in Room 410B. This year's talk is entitled "What Does the Physician need from the Andrology Laboratory?" presented by Christopher L.R. Barratt, Ph.D. Among the topics to be discussed are high quality comprehensive semen analysis, diagnostic and prognostic information and cost effective sperm function and genetic testing. Tickets are available for $25.00 USD and may be purchased by contacting the Congress Office. You may also buy tickets on-site at the main registration and information desk.

Slide Preview

Room 409C located in the west wing of the convention floor, will be available from 7:00-18:00 Saturday through Tuesday, for previewing slides and preparing presentations. A limited number of slide carousels will be available, therefore, presenters are encouraged to bring their own.

Hotel Accommodations

In order to secure your accommodations in one of the official Congress hotels (the Inter-Continental Hotel: 360 Rue Saint-Antoine Street West; the Wyndham Hotel: 1255 Jeanne-Mance Street) at exclusive room rates offered only to VIIth ICA participants, please complete and return a Congress housing form to the Montréal Housing Bureau with a check, money order, or the appropriate credit card information. Discounted room rates are being offered at the Wyndham Hotel for $160 CDN and at the Inter-Continental Hotel for $262 CDN. There is also space available at McGill University Residences (3935 University Street) for $43 CDN a night. Housing forms can be downloaded from the Congress website (www.isa2001.org) or requested from the Congress office.

We can only guarantee a room at this rate until April 30, 2001, therefore, we urge you to make your reservations as soon as possible. Please contact the Montréal Housing Bureau with questions. They can be reached by post (1555 Peel Street, Suite 600, Montréal, QC, Canada H3A 3LB), by phone (514- 844-0848), by fax (514-844-6771) or by email (reservation@tourisme-montreal.org).

Exhibits

An extensive exhibit hall featuring products and information relevant to reproductive and men's health will be open from 10:00 to 18:00 on Saturday, Sunday and 10:00 to 16:00 Monday. Specifically, companies in the business of pharmaceuticals, diagnostics, medical and scientific equipment, biotechnology research and development, publishers of medical and scientific books, journals and electronic media will exhibit at this important world Congress. The 10:30-11:00 and 15:00-15:30 health breaks will be held in the exhibit hall (Convention Center, Room 407B).

Message Center

Thanks to the generous support of N.V. Organon, a Message Center will be provided for delegates during the Congress.
General Information

Poster Sessions
Poster sessions will be held Saturday, Sunday and Monday between 11:00-12:30 and 13:30-15:00 in the Convention Center, Room 407B. Poster boards will be 4 ft. high x 8 ft. wide or 120 cm x 240 cm. Please bring your own pushpins to mount your poster(s). Presenters should put up their posters the morning of their assigned session and stand near their posters during the session to answer questions about their research and findings. Posters must be removed at the end of the day at 18:30. Any poster remaining the following morning will be removed. The ISA President’s Poster Presentations will be held on Tuesday, June 19, 8:00-9:00 in the Convention Center, Room 411ABC. This special session features fifty abstracts specially selected by the abstract peer review committee, and judged to be the best papers submitted to this year’s Congress.

Additional Information for Non-Canadian Colleagues

About Montréal
Montréal is one of the world’s most fascinating cosmopolitan cities located at the foot of Mount Royal. It is a metropolis where rivers and cultures come together. A unique French and English heritage flourished over the years, offering to visitors the distinct charm of European traditions. Montréal is a fun and safe city to walk in. Downtown Montréal is rich in shops, galleries and museums.

Old Montréal provides an opportunity to relive great moments in the city’s history and to expose the newcomer to a rich architectural heritage. The downtown area is attractive and diverse. At the foot of the city’s towering skyscrapers, visitors will discover beautiful churches and magnificent old buildings.

A walk along the Boulevard St-Laurent is like a short walk in Europe, where Greek, Portuguese, German and Italian foods and merchandise are found abundantly. Montréal has several museums, a botanical garden, a safe and easy subway system, and literally thousands of shops, restaurants, movie and stage theaters, connected via an extensive “underground city”. Accessibility from Europe, Asia, South America, and Africa is remarkably easy.

The beginning of the summer is the most beautiful time in Montréal and an average daytime temperature ranges from 18°C (64°F) to 28°C (83°F).

Currency
Visitors to Canada are encouraged to exchange funds for Canadian dollars at the airport upon arrival, or at a bank or foreign currency exchange outlet (located across from the Convention Center at the corner of St-Urbain & Réne Lévesque). Regular bank hours are 10:00-15:00 Monday - Friday. The Canadian dollar has been trading between $0.63-0.68 US for the past two years. You will have to verify its value at the time of the Congress. Please note however, that Congress registration fees are calculated in US dollars and should be paid in US dollars. Therefore, please plan on having some US dollars, a credit card or travelers checks, to pay for any on-site registrations, additional banquet tickets or unpaid balances to your Congress registration fees.

Health Insurance
Access to Canada’s health care services is very costly for non-residents. Therefore, it is recommended that participants arrange health and accident insurance prior to traveling to the Congress.

Airports
Montréal is accessible by two airports: Dorval (U.S., intercontinental and domestic flights) and Mirabel (charter flights). A typical taxi fare between Dorval and downtown Montréal is about $25 CDN, while traveling from Mirabel to downtown Montréal is approximately $65 CDN. All departures from Dorval are subject to $10 CDN airport improvement tax.

Passports and Visas
U.S. residents may enter Canada with proof of citizenship. A visa is required for residents of certain countries. Please consult the Canadian Embassy, High Commission or Consulate in your country for further details and instructions.
Transportation into Montréal

The Inter-Continental Hotel (360 Rue Saint-Antoine Ouest) and Wyndham Hotel (1255 Rue Jeanne Mance) are in downtown Montréal, 10 miles or 16 km, from Dorval Airport, and 0.5 mile from the Central train station.

By air: The best and most economical way to the Hotels from Dorval Airport is to take the Aerobus (departs every 20 minutes) from the airport to downtown Montréal ($11 CDN). Make sure to inform the dispatcher that you are going to the Inter-Continental or to the Wyndham Hotel. A courtesy shuttle will take you to the door of these hotels. With a reservation, the same shuttle will pick you up from the hotel at the date and time of your departure. Please note that a taxi ride from Dorval Airport could cost as much as $25 CDN.

By train: Montréal is served by trains departing from Washington D.C. and New York, as well as from all across Canada. The train stations (Central and Windsor) are just below boulevard René Lévesque, between University Street and Drummond Street. A short taxi ride from the station to the hotels ought to cost no more than $7 CDN, including baggage.

By bus: Buses arrive at the terminus at 505 Maisonneuve East (corner of Berri). Take a taxi directly to the hotels (about $7 CDN). For the Wyndham Hotel you may also take the Métro (direction Angrignon) to Place des Arts station and from there, walk to the hotel (about 1 block). For the Inter-Continental Hotel you may also take the Métro (direction Côte-Vertu) to Place d’Armes station, and from there walk to the hotel (about 1 block).

By car: First, some notes on Québec driving regulations: Wearing a seat belt is mandatory. All road signs are only in French. It is not legal to make a right turn on a red light. A flashing green light indicates that a left is protected against oncoming traffic. A left arrow means that a left turn is permissible but not necessarily protected.

From the South: Take Quebec 15 Nord (North) to Pont Champlain-Montréal (exit 53). Get into the Right lane on Pont Champlain (bridge). Immediately after the bridge, bear right to Autoroute Bonaventure-Centre Ville. Stay in center lane and follow the signs to Centre Ville-University. The autoroute becomes University Street (rue Université). If you are going to the Inter-Continental Hotel, continue up University Street to Saint-Antoine Street (third light), make a right, and the hotel is two blocks further on the right side. If you are going to the Wyndham hotel, continue up University Street (about four blocks) to boulevard René Lévesque, make a right and continue up to Jeanne-Mance Street (three blocks), make a left, and the hotel on the right side.

From the West: Take Quebec 20 Est (East). Follow signs to Montréal-Centre Ville. Do not bear right toward Montréal Ouest (West). Approaching the city, follow Ville Marie-Tunnel. Move to the right lane and exit at rue de la Montagne/rue St.-Jacques (exit 4). Keep to the left, go all the way to the top of the hill and turn right at rue St.-Antoine. The Inter-Continental Hotel is 1.5 miles following this Street. For the Wyndham Hotel you must proceed as directed to rue St.-Antoine and turn left on the corner of Jeanne-Mance. The Wyndham Hotel is four blocks away.

From the East: Take Quebec 20 Ouest (West). At exit 90 follow signs to Pont Jacques Cartier (avoid the Tunnel Louis H. Lafontaine). Take exit 8 to Pont Jacques Cartier (bridge). Cross the bridge and take the first exit right to downtown. Turn right on Viger Street and turn left on Alexandre. After one block turn right on St.-Antoine. The Inter-Continental Hotel is 2 miles away. To go to the Wyndham Hotel cross the Pont Jacques Cartier (bridge) and take the first exit right to downtown. Turn right on boulevard Réné Lévesque and go straight ahead for approximately 2 miles. At the corner of Jeanne-Mance turn right. The hotel is on the right side.

Via Quebec 10: Continue across Pont Champlain (bridge) and proceed as directed in, from the South.
Serono Award Lectureship

John D. Gearhart, Ph.D., is currently Professor in the Departments of Medicine, Gynecology and Obstetrics, Comparative Medicine, Physiology, and Biochemistry at Johns Hopkins University. He is also Director of Research and Director of the Division of Developmental Genetics in the Department of Gynecology and Obstetrics. After completing his Ph.D. in Genetics at Cornell University in 1970, he spent five years, first as a postdoctoral fellow and then as a research associate, at the Institute for Cancer Research in Philadelphia working with Dr. Beatrice Mintz, a pioneer in the development of chimeras to understand cell lineage. In 1975, he went to the University of Maryland as an Assistant Professor and moved across town in 1980 to Johns Hopkins University where he was appointed as an Associate Professor of Pediatrics, Cell Biology and Anatomy, and Gynecology and Obstetrics. Dr. Gearhart is the recipient of several awards, including the Basil O'Connor Starter Research Award from the March of Dimes Birth Defects Foundation and the Joseph P. Kennedy Scholar Award in mental retardation.

Dr. Gearhart has published over 60 peer reviewed articles. He started his career as an independent scientist by studying the totipotency mouse teratocarcinoma cells. He has made several contributions to our understanding of the genetic basis of models for Down's Syndrome, Alzheimer's disease, and germ cell specific gene expression. In 1998, he published a breakthrough article in the Proceedings of the National Academy of Sciences on the "derivation of pluripotent stem cells from cultured human primordial germ cells". The impact of this research is far reaching and is likely to have dramatic consequences in many fields of science and medicine. Dr. Gearhart’s description of these unique cells is that they "... will rapidly let us study human processes in a way we couldn't before. Instead of having to rely on mice or other substitutes for human tissues, we'll have a unique resource that we can start applying to medicine."

Serono Lectureship Recipients

<table>
<thead>
<tr>
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<tr>
<td>1980</td>
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<td>1981</td>
<td>Pierre Soupart</td>
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<td>Maria L. Dufau</td>
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<td>Roger Guillemin</td>
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<td>1989</td>
<td>Frank S. French</td>
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<td>David C. Page</td>
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<td>Leroy Hood</td>
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<td>Norman B. Hecht</td>
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<td>Jurrien Dean</td>
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<td>Neal First</td>
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<td>Bert O'Malley</td>
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The Serono Lectureship is sponsored by Serono Laboratories, Inc.

Distinguished Andrologist Award Recipients

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<tr>
<td>1976</td>
<td>Roy O. Greep</td>
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<td>M.C. Chang</td>
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<td>Robert J. Hotchkiss</td>
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<td>1979</td>
<td>Thaddeus Mann</td>
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<td>Alexander Albert</td>
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<td>1982</td>
<td>Eugenia Rosemburg</td>
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<td>Kristen B.D. Eik-Nes</td>
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<td>Mortimer B. Lipsett</td>
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<td>Robert H. Foote</td>
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<td>1986</td>
<td>Alfred D. Jostr</td>
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<td>Emil Steinberger</td>
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<td>Yves W. Clermont</td>
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<td>Anna Steinberger</td>
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<td>Richard J. Sherins</td>
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<td>Rupert P. Amann</td>
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<td>Brian P. Setchell</td>
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<td>Ryuzo Yanagimachi</td>
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<td>1999</td>
<td>Richard D. Amelar</td>
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<tr>
<td>2000</td>
<td>Bayard T. Storey</td>
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</table>

The Distinguished Andrologist Award is sponsored by the American Society of Andrology.
Distinguished Andrologist Award 2001

Frank S. French, M.D. is Professor of Pediatrics and Director of the Laboratories for Reproductive Biology at the University of North Carolina School of Medicine at Chapel Hill. He received a B.A. degree from the University of Kansas in 1951 and M.D. from the University of Rochester in 1956.

Following residency training in pediatrics at Cleveland City Hospital and North Carolina Memorial Hospital, he was a Fellow in Pediatric Endocrinology and Biochemistry from 1962-1966 at the University of North Carolina School of Medicine at Chapel Hill. In 1971-1972 he was a visiting scientist at the Karolinska Institute, Stockholm, Sweden. Dr. French and associates discovered rat androgen binding protein (ABP), its secretion by Sertoli cells into testicular fluid and concentration in the lumen of proximal epididymis. ABP was used as a marker in studies on FSH and androgen regulation of Sertoli cell function. ABP was later purified and in 1986 cloning and comparison with the sequence of sex hormone binding globulin indicated that the two proteins are encoded by a single gene. His early studies on the human androgen insensitivity syndrome reported in 1965 indicated a target cell defect in androgen response. This was followed by the demonstration of a defect in the nuclear accumulation of androgen in the androgen insensitive Stanley-Gumbrell rat. In subsequent years his research centered on characterization of the androgen receptor and its regulation of male reproductive function. Several androgen dependent proteins including rat acidic epididymal glycoprotein and the rat prostate proteins prostatein, 20 kDa protein and transglutaminase were purified and their structures determined by cloning and sequencing. In 1988 Dr. French and colleagues cloned the AR cDNA, localized the gene on the X-chromosome and identified AR gene mutations that cause androgen insensitivity in humans and rodents. Sequencing the intron-exon junctions of the AR gene opened the way for many investigators to identify AR gene mutations associated with human androgen insensitivity and prostate cancer. Analysis of mutant ARs yielded important insight into structure-function relationships of AR. More recent studies have focused on response elements in AR regulated genes, AR coregulators of gene transcription in testis and the role of AR in recurrent prostate cancer. Dr. French and collaborators are also utilizing molecular cloning and computer analysis of databases to identify and characterize human and monkey epididymis specific proteins involved in sperm maturation and fertilization.

In 1982 Dr. French assumed leadership of the Laboratories for Reproductive Biology, a center that continues to foster research on the male reproductive tract, fertilization and implantation. The Laboratories for Reproductive Biology is now part of the Specialized Centers Program for Research in Reproduction of the National Institute of Child Health and Human Development and includes training programs supported by NICHD, The Andrew W. Mellon Foundation and the Fogarty International Center of NIH. Dr. French was a member of the NIH Reproductive Biology Study Section from 1975-1979 and the Biochemical Endocrinology Study Section from 1991-1995. He is currently the recipient of a MERIT award from the National Institute of Child Health and Human Development. In 1989 he was the Serono Award Lecturer of the American Society of Andrology.

Dr. French is widely praised by his peers for his gracious nature and his unique ability to succeed in both cutting edge research and clinical practice. "The ripple effects from Dr. French's discoveries have impacted many fields including andrology, reproductive biology, prostate cancer, developmental biology and others." "Dr. French has distinguished himself by a long and successful career as both a practicing Pediatric Endocrinologist and as a first-rate research scientist in male hormone action."

"Dr. French was in the research team that cloned androgen binding protein and the androgen receptor, arguably two of the most important achievements in the basic science end of andrology in the last 20 years. For his excellent advancement of our understanding of male reproduction, the Society is proud to present Dr. Frank S. French with the 2001 Distinguished Andrologist Award.
Gail S. Prins, Ph.D. is the 2001 recipient of the Distinguished Service Award. She is a Professor of Physiology in Urology at the University of Illinois at Chicago. She also holds appointments there in the Department of Physiology, and Obstetrics and Gynecology. Dr. Prins received her Ph.D. in Physiology from the University of Illinois in 1979 under the mentorship of Dr. Larry Zaneveld. She then completed a postdoctoral fellowship in Urology at Northwestern University. In 1983, Dr. Prins became an Assistant Professor at the University of Chicago and Director of the In Vitro Fertilization and Andrology Laboratories at Michael Reese Hospital. In 1991, Dr. Prins joined the Department of Obstetrics and Gynecology at the University of Illinois as an Associate Professor and jointly directed the Reproductive Biology Laboratories at Michael Reese Hospital. In 1996, Dr. Prins moved to her current position in the Department of Urology where she is Director of the University Andrology Laboratories.

Dr. Prins is recognized for her research in basic and applied studies of male reproduction. Her major research work, which is funded by the NIH and EPA, involves studies on the prostate gland with an emphasis on hormonal control of prostatic development, growth and function. Her clinical research interests pertain to fertility analysis in the subfertile male and cryobiology of human spermatozoa. Dr. Prins has published 100 manuscripts on these topics.

Dr. Prins has been an active member of the ASA since 1978 and has attended every annual meeting of ASA since its inception. She has served the ASA as a member and Chair of the Student Affairs Committee (1987-1990), as well as a member of the Membership Committee (1988-1990), Andrology Laboratory Committee (1992-1993), Nominating Committee (1992-1993), Long-Range Planning Committee (1994-1996) and Program Committees for the 2000 and 2002 annual meetings. Dr. Prins was a founder of the Women in Andrology within the ASA in 1991 and established its

ASA Distinguished Service Award Recipients

<table>
<thead>
<tr>
<th>Year</th>
<th>Name</th>
<th>Year</th>
<th>Name</th>
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<tr>
<td>1994</td>
<td>C. Alvin Paulsen</td>
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<td>Rupert Amann</td>
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<td>Andrzej Bartke</td>
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<td>David W. Hamilton</td>
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<td>Philip Troen</td>
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<td>1997</td>
<td>Marie-Claire Orgebin-Crist</td>
<td>2000</td>
<td>Bernard Robaire</td>
</tr>
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</table>

The Distinguished Service Award is sponsored by the Genetics & IVF Institute.

name, stated goals and initial membership list. Dr. Prins was a member of the Executive Council from 1990-1993 and subsequently served as ASA Treasurer from 1993-1996. During her tenure as Treasurer, Dr. Prins established the accounting system for ASA finances in two separate business offices, established investment accounts for ASA’s revenue and Endowment Funds, established insurance policies and guided ASA through its fiscal growth during those years. She has been a member of the Finance Committee since 1993 and is currently Chair of that committee where she is responsible for establishing the ASA budgets and overseeing fiscal policies for the Society. Dr. Prins served as an Associate Editor of the Journal of Andrology between 1992-1997 and currently reviews manuscripts for the Journal on an ad hoc basis. She was an unsuccessful candidate for ASA President in 1997 and 1999.

Dr. Prins is committed to the long-range future of ASA and maintaining its financial solvency and growth. She envisions a fiscally strong society with a large endowment which will allow for the future development of a Research and Education Foundation in Andrology.

Dr. Prins’ dedication to our Society is deeply appreciated by her peers, as expressed in their nomination letters. ”It is her dedication to the financial welfare of the Society that deserves recognition. I cannot imagine how many hours she has spent in dealing with the Society’s budget, with the Society’s investments and it’s day-to-day finances”. ”Perhaps even more amazing than her service to the Society has been her collegiality. She has produced the best antibody to the androgen receptor and has willingly shared it with anyone who asked for it, even some of her competitors in prostate research”. For her diligent service and scientific collegiality in the field of Andrology, the Society proudly presents Dr. Gail S. Prins with the 2001 Distinguished Service Award.
Young Andrologist Award 2001

Jacquetta M. Trasler is the recipient of the 2001 Young Andrologist Award. She is an Associate Professor in the Departments of Pediatrics, Human Genetics and Pharmacology & Therapeutics at McGill University and Director of the Developmental Genetics Laboratory at the Montréal Children’s Hospital Research Institute, also at McGill. She obtained her M.D. from McGill University in 1980, followed by two years of clinical training, including one year of Obstetrics and Gynecology. In 1987, she received a Ph.D. from McGill University under the supervision of Dr. Bernard Robaire, studying the male-mediated developmental effects of anti-cancer drugs. After postdoctoral training in molecular biology with Dr. Norman Hecht at Tufts University in Boston, Dr. Trasler joined the faculty at McGill University in 1990. She is Co-Leader of the Medical Genetics and Genomics Axis of the McGill University Health Centre Research Institute and teaches pharmacology and genetics to undergraduate, graduate and medical students. At McGill, Dr. Trasler has mentored a number of pre-medical and graduate students, and is Director of the McGill University M.D./Ph.D. Program.

As a scientist, Dr. Trasler’s research interests focus on the molecular and developmental regulation of gene expression in the testis and the implications for the resulting embryos, with specific interests in DNA methylation and genomic imprinting and the molecular and cellular targets for drug effects on male germ cells. As an independent investigator, she has won several career awards, her latest being the Canadian Institutes of Health Research (CIHR) Scientist Award which she holds concurrently with a Scholarship from the Fonds de la recherche en santé du Québec. Her research is supported by several competitive funding agencies including the CIHR, the Cancer Research Society, the Toxic Substances Research Initiative, the Canadian Foundation for Innovation and the Fonds pour la formation de chercheurs et l’aide à la recherche. Dr. Trasler’s research contributions have been recognized through numerous invitations to present her studies at meetings including the American Society of Andrology, Society for the Study of Reproduction, the North American Testis Workshop and Gordon Research Conferences. She has also been asked to write review articles on topics such as DNA methylation in germ cells for Seminars in Cell and Developmental Biology, and the reproductive consequences of paternal exposure to toxins in Teratology. Dr. Trasler has contributed to over 37 refereed publications and 3 book chapters during her career.

Dr. Trasler has contributed to the American Society of Andrology as an Executive Council Member and has previously served as a member of the Local Arrangements, Publications, Nominating, and Education Policy Committees. She has also served on the Editorial Board of the Journal of Andrology, and was a co-editor of the 1995 ASA Handbook of Andrology.

Dr. Trasler has been struck by the time, energy and enthusiasm the more senior members of ASA dedicate to encourage and mentor trainees and junior faculty members, and by the fact that junior members are given the opportunity to be actively involved in all aspects of the Society early in their careers.

Dr. Trasler is highly praised by her peers in the ASA. “She has established herself as a world class investigator in the field of germ cell biology.” Dr Trasler has established herself within the last decade as one of the key investigators in the area of DNA methylation in germ cells.” “She is a remarkable role model for younger women members of the Society.” For her research accomplishments and enthusiastic work for the ASA, our Society is proud to present Dr. Jacquetta M. Trasler with the 2001 Young Andrologist Award.

Young Andrologist Award Recipients

<table>
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<tr>
<th>Year</th>
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<tr>
<td>1982</td>
<td>L.J.D. Zaneveld</td>
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<td>William B. Neaves</td>
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<td>Barry T. Hinton</td>
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<td>Luis Rodriguez-Rigau</td>
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<td>Gary R. Klinefelter</td>
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<td>Robert Chapin</td>
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<td>Christopher J. De Jonge</td>
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<td>Stuart E. Ravnik</td>
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<td>Matthew P. Hardy</td>
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The Young Andrologist Award is sponsored by the Texas Institute of Reproductive Medicine and Endocrinology, P.A.
New Investigator Award 2001

The New Investigator Award will be given to the trainee delegate with the best abstract and research presentation at the VIIth International Congress of Andrology. The award encourages trainee members to submit and present their best work and to contribute to the scientific excellence of the Society.

The recipient of the 2001 New Investigator Award will be announced Tuesday, June 19 at 11:30 am in Room 407C of the Convention Center.

New Investigator Award Recipients

<table>
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<tr>
<th>Year</th>
<th>Name</th>
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<td>1984</td>
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<td>Randall S. Zane</td>
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<td>Tracy L. Rankin</td>
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<td>Donna O. Bunch</td>
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<td>John Kirby</td>
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The New Investigator Award is sponsored by the West Michigan Reproductive Institute, P.C.

Course Objectives & CME Credit Information

VIIth International Congress of Andrology

Following the scientific program the participant should be able to:

- Recognize the new advances in prostate and testicular cancer
- Describe the role of androgens and androgen therapy in ageing
- Know the new developments on male contraception, male sexual dysfunction, sexually transmitted diseases, clinical genetics and population variation, and on various epidemiological aspects of andrology
- Understand the application of new technological developments in andrology such as DNA chips, high throughput screening, drug discovery and design, molecular genetic diagnosis and transgenic mouse technology

This event is approved for up to 32 credits by the Center for Continuing Medical Education ("CME"). The Center for CME, Faculty of Medicine, McGill University is fully accredited by the Committee of Canadian Medical Schools (CACMS), as well as, the Accreditation Council for Continuing Medical Education (ACCME) of the United States, to sponsor continuing medical education for physicians.

This program meets the accreditation criteria of the College of Family Physicians of Canada for MAINPRO-M1 credits. Members of the American Academy of Family Physicians are eligible to receive credit hours for attendance at this meeting due to the reciprocal agreement with the College of Family Physicians of Canada.

This event is an accredited group learning activity as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada.
Course Objectives & CME Credit Information

The Center for CME, Faculty of Medicine, McGill University designates this activity for Category 1 credit towards the AMA Physicians Recognition Award up to the maximum number of credit hours noted above. Each physician should claim only those hours of credit that he/she actually spent at the education activity.

How To Obtain Credit

Physicians, as well as, Nurses and other Health Care Professionals are eligible for CME and AMA credits. To obtain credits, it is necessary to apply in person at the McGill CME desk, located adjacent to the main registration and information desk, in the lobby of the convention center. The desk will be staffed on Friday, June 15, from 13:00-18:00, on Saturday, June 16- Monday, June 18 from 7:00-18:00 and Tuesday, June 19, from 7:00-12:00. To obtain a CME attestation, participants must personally sign-in once a day for every day they attend the educational activity.

Please Note: You must apply in person for CME credits during the conference. They CANNOT be obtained from writing to the meeting's secretariat or the CME office after the event.

Postgraduate Course I

After attending this course the participant will be able to:

- Describe and administer the new medical therapies for erectile dysfunction
- List the preventable causes of erectile dysfunction and offer patients strategies to reduce their risk
- Understand molecular strategies to treat erectile dysfunction
- Describe the role of surgical intervention and list success rates for erectile dysfunction
- Identify the appropriate strategy for sperm retrieval in patients with azoospermia
- Describe microsurgical procedures to correct obstructive azoospermia

This event is approved for up to 7 credits by the Center for Continuing Medical Education ("CME"). The Center for CME, Faculty of Medicine, McGill University is fully accredited by the Committee of Canadian Medical Schools (CACMS), as well as, the Accreditation Council for Continuing Medical Education (ACCME) of the United States, to sponsor continuing medical education for physicians.

This program meets the accreditation criteria of the College of Family Physicians of Canada for MAINPRO-M1 credits. Members of the American Academy of Family Physicians are eligible to receive credit hours for attendance at this meeting due to the reciprocal agreement with the College of Family Physicians of Canada.

This event is an accredited group learning activity as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada.

The Center for CME, Faculty of Medicine, McGill University designates this activity for Category 1 credit towards the AMA Physicians Recognition Award up to the maximum number of credit hours noted above. Each physician should claim only those hours of credit that he/she actually spent at the education activity.

How To Obtain Credit

Physicians, as well as, Nurses and other Health Care Professionals are eligible for CME and AMA credits. To obtain credits, it is necessary to apply in person at the McGill CME desk, located in the Wyndham Hotel, near the main registration area. The desk will be staffed on Thursday, June 14, from 7:00-13:00. To obtain a CME attestation, participants must personally sign-in.

Please Note: You must apply in person for CME credits during the conference. They CANNOT be obtained from writing to the meeting's secretariat or the CME office after the event.
Course Objectives & CME Credit Information

Postgraduate Course II

After attending this course the participant will be able to:

- Understand the philosophy and purpose of the WHO manual, including its role in defining international minimum standards for semen analysis and other laboratory tests in the work-up of the infertile couple. Understand the principles governing the development and introduction of new procedures for future editions of the manual with particular reference to sperm functions tests

- Identify the existence and significance of genomic endpoints of sperm function, with particular reference to the male contribution to embryonic development

- Recognize biochemical endpoints of sperm physiology and practical issues governing their assessment and potential future applications in assessing sperm functional competence as well as in causing sperm dysfunction

- Understand the application of CASA technology to the laboratory assessment of spermatozoa, with particular reference to the derivation of kinematic measures of sperm motility (including the significance of the inherent limitations of CASA technology in this regard) and how sperm kinematics can be used in (a) the diagnosis and management of male factor infertility and (b) reproductive toxicology

- Need for international standardization, training programs, quality control and external quality assurance programs in semen analysis, using Tygerberg Strict Criteria morphology as an example

- Understand the factors that govern the selection and application of optimized sperm preparation methods with special reference to the usefulness of prepared spermatozoa in assisted reproductive technology.

This event is approved for up to 6 credits by the Center for Continuing Medical Education ("CME"). The Center for CME, Faculty of Medicine, McGill University is fully accredited by the Committee of Canadian Medical Schools (CACMS), as well as, the Accreditation Council for Continuing Medical Education (ACCME) of the United States, to sponsor continuing medical education for physicians.

This program meets the accreditation criteria of the College of Family Physicians of Canada for MAINPRO-M1 credits. Members of the American Academy of Family Physicians are eligible to receive credit hours for attendance at this meeting due to the reciprocal agreement with the College of Family Physicians of Canada.

This event is an accredited group learning activity as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada.

The Center for CME, Faculty of Medicine, McGill University designates this activity for Category 1 credit towards the AMA Physicians Recognition Award up to the maximum number of credit hours noted above. Each physician should claim only those hours of credit that he/she actually spent at the education activity.

How To Obtain Credit

Physicians, as well as, Nurses and other Health Care Professionals are eligible for CME and AMA credits. To obtain credits, it is necessary to apply in person at the McGill CME desk, located in the Wyndham Hotel. The desk will be staffed on Wednesday, June 20, from 7:00-13:00. To obtain a CME attestation, participants must personally sign-in.

Please Note: You must apply in person for CME credits during the conference. They CANNOT be obtained from writing to the meeting's secretariat or the CME office after the event.
Thursday, June 14

The Congress offers two postgraduate courses. The first, "RECENT ADVANCES IN CLINICAL ANDROLOGY" will be held June 14 and the second, "Sperm Function and WHO Criteria" on June 20.

POSTgraduate COURse I: RECENT ADVANCES in CLINICAL ANDROLOGY

WYNDHAM HOTEL
Alfred Rouleau Room

Sponsored by Bayer Corporation, Pharmaceutical Division

This Course will include presentations on the pharmacological management and prevention of erectile dysfunction, gene therapy for erectile dysfunction and state-of-the-art lectures on the surgical management of erectile dysfunction and obstructive azoospermia among others.

Chairs: Jonathan P. Jarow, M.D. (USA); Serge Carrier, M.D., F.R.C.S.(C) (Canada)

07:00-8:00 REGISTRATION

07:50-8:00 WELCOMING REMARKS

MEDICAL MANAGEMENT OF ERECTILE DYSFUNCTION
Jacob Rajfer, M.D., Harbor-UCLA Medical Center (USA)

08:00-8:45 PHYSIOLOGY AND PATHOPHYSIOLOGY OF ERECTILE DYSFUNCTION
Arthur L. Burnett, M.D., Johns Hopkins Hospital (USA)

08:45-9:15 UPDATE ON PHARMACOLOGIC THERAPIES FOR ERECTILE DYSFUNCTION
Alvaro Morales, M.D., Queen’s University (Canada)

09:15-10:00 FUTURE FRONTIERS: GENE THERAPY FOR ERECTILE DYSFUNCTION
Jacob Rajfer, M.D., Harbor-UCLA Medical Center (USA)

10:00-10:15 HEALTH BREAK

SURGICAL THERAPY FOR ERECTILE DYSFUNCTION
Serge Carrier, M.D., F.R.C.C. (C), McGill University (Canada)

10:15-10:45 PENILE PROSTHETICS IN THE MANAGEMENT OF ERECTILE DYSFUNCTION AND PEYRONIE’S DISEASE Steven K. Wilson, University of Arkansas (USA)

10:45-11:15 ARTERIAL RECONSTRUCTIVE SURGERY
Jonathan P. Jarow, M.D., Johns Hopkins University School of Medicine (USA)

11:15-11:45 VENOUS LIGATION SURGERY
Eric Wespes, M.D., Universite Libre de Bruxelles (Belgium)
Thursday, June 14

11:45–13:00  LUNCH (on your own)

SPERM RETRIEVAL AND MICRO SURGICAL RECONSTRUCTION
Marc Goldstein, M.D., Cornell University Medical Center (USA)

13:00–13:45  TESTICULAR MAPPING FOR TESE
Paul J. Turek, M.D., University of California, San Francisco (USA)

13:45–14:30  TESTICULAR MICRODISSECTION FOR TESE
Peter N. Schlegel, M.D., Weill Medical College of Cornell, New York Presbyterian Hospital (USA)

14:30–15:15  PERCUTANEOUS EPIDIDYMAL ASPIRATION (PESA)
Ian Craft, F.R.C.S., F.R.C.O.G., London Gynaecology and Fertility Center (UK)

15:15–15:30  HEALTH BREAK

15:30–16:15  VASOVASOSTOMY
Marc Goldstein, M.D., F.A.C.S., Cornell University Medical Center (USA)

16:15–17:00  EPIDIDYMOSVASOSTOMY
Richard E. Berger, University of Washington (USA)

Friday, June 15

Congress Program

09:00–17:00  ASA EXECUTIVE COUNCIL MEETING
Wyndham Hotel, Auteuil Room

16:00–17:00  ISA EXECUTIVE COMMITTEE MEETING
Convention Center, Room 312

18:00–18:30  OPENING CEREMONY
Convention Center, Room 407C
Ilpo T. Huhtaniemi, M.D., Ph.D., ISA President
J. Lisa Tenover, M.D., Ph.D., ASA President
Carlos R. Morales, D.V.M., Ph.D., Local Organizing Committee Chair
Friday, June 15

18:30-19:30  PLENARY LECTURE 1: SERONO LECTURE
Convention Center, Room 407B
Chair: Héctor E. Chemes, M.D., Ph.D. (Argentina)
STEM CELLS, GERM CELL ENGINEERING AND CLONING
John D. Gearhart, Ph.D., Johns Hopkins University School of Medicine (USA)

19:30-20:30  OPENING MIXER
Convention Center, Room 407B

Saturday, June 16

08:00-09:00  PLENARY LECTURE 2
Sponsored by Women in Andrology
Convention Center, Room 407C
Chair: Bernard Robaire, Ph.D. (Canada)
GETTING THE RIGHT GENES AND THE RIGHT CHROMOSOMES: THE GENETICS OF
SPERMATOGENESIS
Mary Ann Handel, Ph.D., University of Tennessee (USA)

09:00-10:30  SYMPOSIUM 1: PROSTATE DISEASE
Convention Center, Room 408A
Chair: Sae Chul Kim, M.D., Ph.D. (S. Korea); Han-Sun Chiang, M.D. (Taiwan)
1. THE PRESENT AND FUTURE OF PROSTATE IMAGING
Martin I. Resnick, M.D., Case Western Reserve University (USA)
2. CHEMOPREVENTION OF PROSTATE CANCER
Claude C. Schulman, M.D., University of Brussels (Belgium)
3. INTERMITTENT ANDROGEN BLOCKADE IN THE TREATMENT OF PROSTATE CANCER
Nicholas Bruchovsky, M.D., Ph.D., British Columbia Cancer Agency (Canada)

09:00-10:30  SYMPOSIUM 2: MALE CONTRACEPTION
Sponsored by the CONRAD Program
Convention Center, Room 408B
Chair: Kirsten Vogelson, Ph.D. (WHO); Kamal Z. Mahmoud, M.D. (Egypt)
1. ANDROGENS AS A SINGLE CONTRACEPTIVE AGENT
Yi Qun Gu, M.D., National Research Institute for Family Planning (P.R.China)
Saturday, June 16

2. ANDROGEN PROGESTIN REGIMENS FOR MALE CONTRACEPTION
   M. Cristina Meriggiola, M.D., Ph.D. University of Bologna (Italy)

3. EPIDIDYMAL APPROACHES TO MALE CONTRACEPTION
   Trevor G. Cooper, Ph.D., University of Münster (Germany)

09:00-10:30

SYMPOSIUM 3: GERM CELL TRANSPLANTATION, IN VITRO DEVELOPMENT AND TRANSGENESIS
Convention Center, Room 408C

Chairs: Wolf-Bernhard Schill, M.D., Ph.D. (Germany); Makoto Nagano, Ph.D. (Canada)

1. GERM CELL TRANSPLANTS IN PRIMATES
   Stefan Schlatt, Ph.D., Institut für Reproduktionsmedizin (Germany)

2. IN VITRO MATURATION OF HUMAN SPERMATOGENIC CELLS
   Jan Tesarik, M.D., Ph.D., Laboratoire D'Eylau (France)

3. SPERMATOZOA AS CARRIERS OF FOREIGN DNA
   Masaru Okabe, Ph.D., Osaka University (Japan)

10:30-11:00

HEALTH BREAK
Convention Center, 407B

11:00-12:30

POSTER SESSION I
Sponsored by N.V. Organon
Convention Center, Room 407B

12:30-13:30

WORKSHOP 1: DNA CHIPS
Convention Center, Room 411A

Thomas Hudson, M.D., Montréal Genome Center (Canada)

Robert Sladek, M.D., Montréal Genome Center (Canada)

12:30-13:30

WORKSHOP 2: PROTEOMICS OF THE CELL MAP: HIGH THROUGHPUT 2-D GELS MASS SPECTROMETRY AND BIOINFORMATICS
Convention Center, Room 411B

John J.M. Bergeron, D. Phil., McGill University (Canada)

Michel Desjardins, Ph.D., Université de Montréal, (Canada)

Alexander W. Bell, Ph.D., McGill University (Canada)

12:30-13:30

WORKSHOP 3: INTERSEX DISORDERS: BIOCHEMICAL AND MOLECULAR DIAGNOSIS
Convention Center, Room 411C

Rodolfo A. Rey, M.D., Ph.D., University of Buenos Aires and Research Council (Argentina)
Saturday, June 16

12:30-13:30  JOURNAL OF ANDROLOGY EDITORIAL BOARD LUNCHEON
Convention Center, Room 410B

12:30-13:30  LUNCH (congress box lunch or on your own)

13:30-15:00  POSTER SESSION II
Sponsored by N.V. Organon
Convention Center, Room 407B

15:00-15:30  HEALTH BREAK
Convention Center, Room 407B

15:30-17:00  SYMPOSIUM 4: CLINICAL GENETICS AND POPULATION VARIATION IN ANDROLOGY
Sponsored by ALZA Pharmaceuticals
Convention Center, Room 408A

Chairs: Roger Mieusset, M.D., Ph.D. (France); G.K. Papp, M.D. (Hungary)

1. ETHNIC DIFFERENCES IN MALE REPRODUCTIVE FUNCTION
Ronald S. Swerdloff, M.D., Harbor-UCLA Medical Center (USA)

2. HERITABILITY OF CONTROL OF SERUM ANDROGENS AND PROSTATE GROWTH IN MALE TWINS
A. Wayne Meikle, M.D., University of Utah School of Medicine (USA)

3. POLYMORPHISMS AND MUTATIONS IN GONADOTROPHIN GENES
Tarja Lamminen, Ph.D., University of Turku (Finland)

15:30-17:00  SYMPOSIUM 5: SEXUALLY TRANSMITTED DISEASES
Convention Center, Room 408B

Chair: Hiroyoshi Suzuki, M.D., Ph.D. (Japan); Jun-Kyu Suh, M.D. (Korea)

1. MOLECULAR MECHANISM OF HIV INFECTION AND ITS CLINICAL IMPLICATIONS
Deborah J. Anderson, Ph.D., Harvard Medical School (USA)

2. GLOBAL BURDEN OF PREVENTABLE STDs
Caroline Ryan, M.D., M.P.H., National Center for HIV, STD and TB Prevention (USA)

3. EPIDEMIOLOGICAL SURVEY OF STD/HIV IN JAPAN
Yoshiaiki Kumamoto, M.D., Ph.D., Japanese Foundation of Sexual Health Medicine (Japan)
Saturday, June 16

15:30-17:00 SYMPOSIUM 6: ANIMAL MODELS OF MALE INFERTILITY

Sponsored by: U.S. National Institutes of Health:
National Institute of Child Health and Human Development; National Institute of Aging;
National Institute of Diabetes and Digestive and Kidney Diseases
Convention Center, Room 408C

Chairs: Richard Sharpe, Ph.D. (UK); Jacquetta M. Trasler, M.D., Ph.D. (Canada)

1. DNA REPAIR MECHANISMS AND THE UBIQUITIN SYSTEM IN SPERMATOGENESIS
J. Anton Grootegoed, Ph.D., Erasmus University Rotterdam (The Netherlands)

2. HYBRID STERILITY 6 LOCUS: A MODEL SYSTEM FOR MOTILITY DEFECTS
Stephen Pilder, Ph.D., Temple University School of Medicine (USA)

3. TESTICULAR DEGENERATION IN BCLW-DEFICIENT RATS
Grant R. MacGregor, D.Phil., Emory University School of Medicine (USA)

17:00-18:00 PLENARY LECTURE 3: ERNST SCHERING RESEARCH FOUNDATION LECTURE

Convention Center, Room 407C

Chair: Iipo T. Huhtaniemi, M.D., Ph.D. (Finland)

ANDROGEN ACTION: OLD VIEWS, NEW INSIGHTS AND UNSOLVED MYSTERIES
Albert Brinkmann, Ph.D., Erasmus University Rotterdam (The Netherlands)

18:00-18:15 PRESENTATION OF THE DISTINGUISHED ANDROLOGIST AWARD

Sponsored by American Society of Andrology

Convention Center, Room 407C

18:30-19:30 STUDENT MIXER

Convention Center, Room 311

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Sunday, June 17

08:00-09:00 PLENARY 4

Sponsored by Lilly ICOS

Convention Center, Room 407C

Chair: Christina Wang, M.D. (USA)

ADVANCES IN MALE SEXUAL DYSFUNCTION
Wayne J.G. Hellstrom, M.D., Tulane University Medical Center (USA)
Sunday, June 17

Congress Program

09:00-10:30 SYMPOSIUM 7: TESTICULAR CANCER
Convention Center, Room 408A

Chairs: Arnold M. Belker M.D. (USA); Robert W. Hudson, M.D., Ph.D. (Canada)

1. BIOLOGY OF CARCINOMA IN SITU, THE STEM CELL-LIKE PRECURSOR OF TESTICULAR CANCER
Ewa Rajpert-De Meyts, M.D., Ph.D., Copenhagen University Hospital (Denmark)

2. TESTICULAR CANCER AND REPRODUCTIVE FAILURE OF THE GONADS
Henrik Møller, M.D.Sc., Thames Cancer Registry, King's College (UK)

3. TESTICULAR BIOPSIES AND THE SEARCH FOR EARLY TESTICULAR CANCER
Klaus Peter Dieckmann, M.D., Urologische Abteilung Albertinen-Krankenhaus Hamburg (Germany)

09:00-10:30 SYMPOSIUM 8: CELL SURVIVAL AND DEATH
Convention Center, Room 408B

Chairs: Barry R. Zirkin, Ph.D. (USA); Dimitri Adamopoulos, M.D. (Greece)

1. REGULATION OF PROGRAMMED CELL DEATH
Michael Hengartner, Ph.D., University of Zurich (Switzerland)

MITOTIC/MEIOTIC CHECKPOINTS AND GERM CELL APOPTOSIS
Josefa Blanco-Rodríguez, M.D., Ph.D., Valladolid University (Spain)

3. ANDROGEN DEPENDENT APOPTOSIS AND SURVIVAL IN THE PROSTATE
Martin P. Tenniswood, Ph.D., University of Notre Dame (USA)

09:00-10:30 SYMPOSIUM 9: PUBLIC HEALTH, EPIDEMIOLOGY ASPECTS OF ANDROLOGY.
Convention Center, Room 408C

Chairs: Eduardo Bustos-Obregón, M.D. (Chile); Fernando Vasquez, M.D. (Colombia)

1. MEASURING MALE FERTILITY, METHODOLOGICAL ASPECTS
Jorn Olsen, M.D., Ph.D., The Danish Epidemiology Science Center, University of Aarhus (Denmark)

OCCUPATIONAL FACTORS AND MALE FERTILITY
Jens Peter Bonde, M.D., Ph.D., Aarhus University Hospital (Denmark)

ENVIRONMENTAL INFLUENCES ON MALE FERTILITY
Alfred Spira, M.D., Ph.D., INSERM-Hôpital de Bicêtre (France)

10:30-11:00 HEALTH BREAK
Convention Center, Room 407B

11:00-12:30 POSTER SESSION III
Sponsored by N.V. Organon
Convention Center, Room 407B
Sunday, June 17

12:30-13:30
LUNCH (congress box lunch or on our own)

12:30-13:30
WORKSHOP 4: DRUG DISCOVERY AND DESIGN
Sponsored by N.V. Organon
Convention Center, Room 411A
H. Uri Saragovi, Ph.D., McGill University (Canada)

12:30-13:30
WORKSHOP 5: GERM CELL TRANSPLANTS
Convention Center, Room 411B
Lonnie D. Russell, M.S., Ph.D. Southern Illinois University School of Medicine (USA)

12:30-13:30
WORKSHOP 6: SPERM RETRIEVAL
Convention Center, 411C
Herman Tournaye, M.D., Ph.D., Centre for Reproductive Medicine, Dutch-Speaking Brussels Free University (Belgium)

12:30-13:30
LABORATORY SCIENCE FORUM LUNCH
Convention Center, Room 410B
WHAT DOES THE PHYSICIAN NEED FROM THE ANDROLOGY LABORATORY?
Christopher L.R. Barratt, Ph.D., Birmingham Women’s Hospital (UK)

13:30-15:00
POSTER SESSION IV
Sponsored by N.V. Organon
Convention Center, Room 407B

15:00-15:30
HEALTH BREAK
Convention Center, Room 407B

15:30-17:00
SYMPOSIUM 10: SPERM-OOCYTE INTERACTION
Convention Center, Room 408A
Chairs: Nuch Tanphaichitr Ph.D. (Canada)
1. THE IMPACT OF OXIDATIVE STRESS ON SPERM FUNCTION AND FERTILITY
   John Aitken, Sc.D., F.R.S.E., University of Newcastle (Australia)
2. THE NATURE OF SPERM CAPACITATION: CURRENT CONCEPTS AFTER 50 YEARS OF RESEARCH
   Janice L. Bailey, Ph.D., Laval University (Canada)
3. SPERM-EGG FUSION
   Kiyotaka Toshimori, M.D., Ph.D., Miyazaki Medical College (Japan)
Sunday, June 17

15:30-17:00

DEBATE: ETHICS IN ANDROLOGY
Convention Center, Room 408B

Chair: Michael E. McClure, Ph.D. (USA)

ICSI MODELS IN PRIMATES
Laura Hewitson, Ph.D., Oregon Health Sciences University (USA)

IS ICSI A SAFE THERAPEUTIC APPROACH FOR SEVERE MALE FACTOR INFERTILITY
H.W. Gordon Baker, M.D., Ph.D., University of Melbourne (Australia)

15:30-17:00

SYMPOSIUM 11: ANDROGEN THERAPY AND AGEING
Sponsored by Watson Pharmaceuticals
Convention Center, Room 408C

Chairs: William Bremner, M.D., Ph.D. (USA); Miguel Díaz García M.D. (Mexico)

1. RISKS AND BENEFITS OF TESTOSTERONE IN OLDER MEN
   J. Lisa Tenover, M.D., Ph.D., Emory University (USA)

2. COMBINATION THERAPY OF GH AND TESTOSTERONE IN OLDER MEN
   Marc R. Blackman, M.D., Clinical Director, National Center for Complementary and
   Alternative Medicine (USA)

3. TESTOSTERONE AND BONE FUNCTION
   Joel Finkelstein, M.D., Massachusetts General Hospital (USA)

17:00-18:00

PLENARY LECTURE 5
Sponsored by American Society of Andrology
Convention Center, Room 407C

Chair: Daniel Franken, Ph.D. (South Africa)

TREATING MALE INFERTILITY BY ART/ICSI
Herman Tournaye, M.D., Ph.D., Centre for Reproductive Medicine, Dutch-Speaking Brussels Free
University (Belgium)

18:00-18:30

PRESENTATION OF THE YOUNG ANDROLOGIST AWARD
AND THE DISTINGUISHED SERVICE AWARD
Sponsored by American Society of Andrology
Convention Center, Room 407C
Monday, June 18

07:00-08:00  ASA PAST PRESIDENTS BREAKFAST
Convention Center, Room 410A

08:00-09:00  PLENARY LECTURE 6
Sponsored by ALZA Pharmaceuticals
Convention Center, Room 407C
Chair: Frederick C.W. Wu, M.D.
The Epidemiology of Hyponadism: It’s Magnitude, Clinical Correlates and
Social Significance
John McKinlay, Ph.D., New England Research Institutes (USA)

09:00-10:30  SYMPOSIUM 12: MALE SEXUAL DYSFUNCTION
Sponsored by American Medical Systems
Convention Center, Room 408A
Chairs: Jose Maria Pomerol, M.D. (Spain); Marcos Paulo P de Castro, M.D., Ph.D. (Brazil)
1. Physiopathology of Male Sexual Dysfunction
Gorm Wagner, M.D., Ph.D., University Hospital, Copenhagen (Denmark)
2. New Advances in the Medical Management of Male Sexual Dysfunction
Arthur L. Burnett, M.D., Johns Hopkins Hospital (USA)
3. Surgical Management of Male Sexual Dysfunction Including Peyronie’s Disease
John P. Mulhall, M.D., Loyola University Medical Center (USA)

09:00-10:30  SYMPOSIUM 13: FSH IN THE MALE
Sponsored by N.V. Organon
Convention Center, Room 408B
Chairs: Malur Ram Sairam, M.D., Ph.D. (Canada); Krzysztof Kula M.D., Ph.D. (Poland)
1. FSH and Spermatogenesis: The Clinical Experience
Eberhard Nieschlag, M.D., Institute of Reproductive Medicine of the University, Münster (Germany)
2. Animal Models for FSH Action
Harry M. Charlton, Ph.D., University of Oxford (UK)
3. Protein Engineering of FSH and Novel Gonadotrophins
Irving Boime, Ph.D., Washington University School of Medicine (USA)
Monday, June 18

Congress Program

09:00-10:30  SYMPOSIUM 14: EPIDIDYMIS
Convention Center, Room 408C

Chairs: Jorge Blaquier, M.D., Ph.D. (Argentina); David Hamilton Ph.D. (USA)

1. REGULATION OF GENE EXPRESSION IN EPIDIDYMIS
   Joel R. Drevet, Ph.D., CNRS/Blaise Pascal University (France)

2. CELL-CELL INTERACTION IN THE EPIDIDYMIS
   Daniel G. Cyr, Ph.D., INRS Institute Armand Frappier, Université de Québec (Canada)

3. PARTICIPATION OF EPIDIDYMAL PROTEIN DE IN SPERM-EGG FUSION
   Patricia S. Cuasnicu, Ph.D., Instituto de Biologia y Medicina Experimental, Buenos Aires (Argentina)

10:30-11:00  HEALTH BREAK
Convention Center, Room 407B

11:00-12:30  POSTER SESSION V
Sponsored by N.V. Organon
Convention Center, Room 407B

12:30-13:30  LUNCH (congress boxed lunch or on your own)

12:30-13:30  WORKSHOP 7: FUTURE TRENDS IN HORMONE ASSAYS
Convention Center, 411A
   Göran Lindstedt, M.D., Ph.D., Gothenburg University (Sweden)

12:30-13:30  WORKSHOP 8: CLINICAL APPROACH TO MOLECULAR GENETIC DIAGNOSIS IN ANDROLOGY
Convention Center, Room 411B
   H.W. Gordon Baker, M.D., Ph.D., University of Melbourne (Australia)

12:30-13:30  WORKSHOP 9: TRANSGENIC TECHNOLOGY
Convention Center, Room 411C
   Stephen H. Plder, Ph.D., Temple University School of Medicine (USA)

12:30-13:30  WOMEN IN ANDROLOGY LUNCHEON: CURRENT ISSUES FACING WOMEN IN SCIENCE
Convention Center, Room 410B
   Catherine Jay Didion, Association for Women in Science, (USA)

13:30-15:00  POSTER SESSION VI
Sponsored by N.V. Organon
Convention Center, Room 407B
Monday, June 18

15:00-15:30  HEALTH BREAK
Convention Center, Room 407B

15:30-17:00  ISA BUSINESS AND GENERAL ASSEMBLY AND AWARDS PRESENTATION
Convention Center, Room 407C
ISA TRAVEL AWARDS
Sponsored by N.V. Organon

17:00-17:45  ASA ANNUAL BUSINESS MEETING
Convention Center, Room 407C

19:00-22:30  CONGRESS BANQUET
Dinner and Dancing on the River Boat "Cavalier Maxim"
Motorcoaches will depart from the Inter-Continental and Wyndham Hotels at 18:15 and shuttle delegates and guests back to those hotels at 22:30.

Tuesday, June 19

08:00-9:30  PRESIDENT’S POSTER PRESENTATIONS
Convention Center, Room 411ABC

09:30-10:00  HEALTH BREAK
Convention Center, Room 407C

10:00-11:30  SYMPOSIUM 15: NON TRADITIONAL MARKERS OF REPRODUCTIVE TRACT FUNCTION
Convention Center, Room 408A
Chair: Robert McLachlan, M.D., Ph.D. (Australia)
1. INHIBIN β AS A SERUM MARKER OF SPERMATOGENESIS
Anna-Maria Andersson, Ph.D., Copenhagen University Hospital (Denmark)
2. AMH AND PREPUBERTAL TESTICULAR FUNCTION
Rodolfo A. Rey, M.D., Ph.D., University of Buenos Aires and Research Council (Argentina)
3. INHIBINS, ACITVINs AND FOLLISTATINS IN PROSTATE FUNCTION
Gail Risbridger, Ph.D., Monash University (Australia)
**Tuesday, June 19**

**Congress Program**

10:00-11:30  **SYMPOSIUM 16: ANDROGENS AND BRAIN**  
*Sponsored by Watson Pharmaceuticals*  
Convention Center, Room 408B

*Chair: Gianni Forti, M.D. (Italy); Ganesan Adaikan, M.D. (Singapore)*

1. **MECHANISMS OF ACTION OF ANDROGENS IN THE BRAIN. HOW, WHERE AND WHEN**  
Robert Handa, Ph.D., Colorado State University (USA)

2. **COGNITIVE FUNCTIONS AND ANDROGENS IN MEN**  
Daryl O'Connor, Ph.D., University of Leeds (UK)

3. **ANDROGENS AND SEXUAL FUNCTION**  
James G. Pfau, Ph.D., Concordia University (Canada)

10:00-11:30  **SYMPOSIUM 17: SPERM STRUCTURE AND FUNCTION**  
Convention Center, Room 408C

*Chair: Carlos Morales, Ph.D., D.V.M. (Canada); Aucky Hinting, M.D., Ph.D. (Indonesia)*

1. **The Cytoskeleton of the Sperm Head**  
Richard Oko, Ph.D., Queen's University (Canada)

2. **The Synaptopemal Complex**  
Christer Höög, Ph.D., Karolinska Institute (Sweden)

3. **GAPD-S: A Unique Glycolytic Enzyme Anchored to the Sperm Fibrous Sheath**  
Deborah A. O'Brien, Ph.D., University of North Carolina at Chapel Hill (USA)

11:30-11:45  **ASA AWARDS PRESENTATION**  
Convention Center, Room 407C

**NEW INVESTIGATOR AWARD**  
*Sponsored by West Michigan Reproductive Institute, P.C.*

**RESEARCH EXCELLENCE AWARD FOR FEMALE TRAINEE/FELLOW**  
*Established by Anna Steinberger, Ph.D., supported by Women in Andrology*

**TRAINEE MERIT AWARDS**  
*Sponsored by American Society of Andrology, Lalor Foundation, and U.S. National Institutes of Health: National Institute of Child Health and Human Development, National Institute of Aging, National Institute of Diabetes and Digestive and Kidney Diseases*

**THOMAS S.K. CHANG TRAINEE TRAVEL FUND AWARDS**  
*Sponsored by American Society of Andrology*

**BURROUGHS-WELLCOME FUND AWARD FOR OUTSTANDING TRAINEE RESEARCH**  
*Sponsored by Burroughs-Wellcome Fund*
Tuesday, June 19

11:45-12:45 AMERICAN UROLOGICAL ASSOCIATION PLENARY DEBATE
Convention Center, Room 407C

Chair: David J. Handelsman, M.D., Ph.D. (Australia)

PROSTATE CANCER SCREENING
Moderator: Claude C. Schulman, M.D., University of Brussels (Belgium)
Fernand Labrie, M.D., Ph.D., Laval University (Canada)
Michael Marberger, M.D., University of Vienna (Austria)

12:45 CONCLUSION
Convention Center, Room 407C

12:45-14:15 ISA EXECUTIVE COMMITTEE MEETING
Convention Center, Room 312

Wednesday, June 20

POSTGRADUATE COURSE II: SPERM FUNCTION AND WHO CRITERIA

WYNDHAM HOTEL, GRAND SALON B

The purpose of the POSTGRADUATE ANDROLOGY LABORATORY COURSE at the ISA 2001 Congress is to educate the participant about aspects of sperm function testing and as they relate to the current and likely future editions of the WHO manual.

Chairs: Christopher J. De Jonge, Ph.D., H.C.I.D. (USA); David Mortimer, Ph.D. (USA)

07:00-08:00 REGISTRATION

08:45-09:15 WHO MANUAL - PHILOSOPHY AND FUNCTION
Geoffrey M.H. Waites, Sc.D., Ph.D., University of Sydney (Australia)
<table>
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<tr>
<th>Time</th>
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<tr>
<td>09:15-10:00</td>
<td>WHO - SPERM FUNCTION TESTING</td>
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<td>Christopher L.R. Barratt, Ph.D., Birmingham Women’s Hospital (UK)</td>
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<td>10:00-10:30</td>
<td>HEALTH BREAK</td>
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<td>10:30-11:15</td>
<td>GENOMIC ENDPOINTS OF SPERM FUNCTION</td>
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<td>Andrew J. Wyrobek, Ph.D., Lawrence Livermore National Laboratory, University of California (USA)</td>
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<td>11:15-12:00</td>
<td>BIOCHEMICAL ENDPOINTS</td>
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<td>Juan Alvarez, M.D., Ph.D., Harvard Medical School (USA)</td>
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<td>12:00-13:30</td>
<td>LUNCH (on your own)</td>
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<td>13:30-14:15</td>
<td>MOTILITY/CASA</td>
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<td>Jacques Auger, M.D., Ph.D., Hôpital Cochin, Paris (France)</td>
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<td>14:15-15:00</td>
<td>INITIATION OF A GLOBAL QUALITY CONTROL SYSTEM FOR SPERM MORPHOLOGY:</td>
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<td>3 YEARS OF EXPERIENCE</td>
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<td>Daniel Franken, Ph.D., Reproductive Biology Research Laboratory</td>
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<td>(South Africa)</td>
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<td>15:00-15:30</td>
<td>HEALTH BREAK</td>
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<td>15:30-16:15</td>
<td>SPERM SELECTION FOR ART</td>
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<td>David Mortimer, Ph.D., Oozoa Biomedical (Canada)</td>
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<td>16:15-17:00</td>
<td>SPERM FUNCTION TEST &quot;WISH LIST&quot; FOR THE NEXT MANUAL</td>
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<td>Christopher J. De Jonge, Ph.D., H.C.L.D., University of Minnesota</td>
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<td>(USA)</td>
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Please note: Even numbered abstracts will be presented in the morning session (11:00 - 12:30),
and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

Epididymis

P1/2-001 MORPHOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL STUDIES ON THE ZONATION
OF GOAT EPIDIDYMIS
G.S. Bilaspuri

P1/2-002 EPIDIDYMAI APPROACHES TO MALE CONTRACEPTION
T.G. Cooper

P1/2-003 CELL-CELL INTERACTIONS IN THE EPIDIDYMIS
D.G. Cyr

P1/2-004 ASSOCIATION OF ZO-1 AND BETA-CATENIN IN THE RAT EPIDIDYMIS
S. DeBellefeuille, D.G. Cyr

P1/2-005 EFFECT OF HYPOTHYROIDISM ON THE MORPHOLOGY OF DUCTUS EPIDIDYMIS
(SCANNING ELECTRON MICROSCOPY STUDY)
A.G. Del Rio, L.A. Palaoro, A.M. Blanco, H. Niepomnische

P1/2-006 SPERM MATURATION IN HUMAN EPIDIDYMIS
V. Eysremendy

P1/2-007 IMMUNOLOCALIZATION OF THE LIPOCALIN EP17 IN THE MOUSE AND RAT EPIDIDYMIS
S. Fouchevourt, J.I. Lareyre, D. Ong, R. Matusik, M-C. Orgein-Crist

P1/2-008 EPIDIDYMAI TISSUE SPECIFICITY AND ANDROGEN DEPENDENCE OF RAT EP2
N.M. Ibrahim, L.G. Young, O. Fröhlich

P1/2-009 FERTILITY OF DIFFERENT STRAINS OF RATS SYMPATHETOMIZED WITH GUANETHIDINE (GLIA)
W.D. Kempinas, S.U. Oliva, D.A.F. Silva

P1/2-010 ABNORMALITIES IN CELLS OF THE TESTIS AND EPIDIDYMIS IN CATHEPSIN A KNOCKOUT MICE
N. Korah, A. D'Azoo, L. Hermo

P1/2-011 ABNORMALITIES IN CELLS OF THE TESTIS AND EPIDIDYMIS IN ADULT MICE WITH CATHEPSIN
A DEFICIENCY
N. Korah, A. D'Azoo, L. Hermo

P1/2-012 EXPRESSION OF THE PROTO-ONCOGENE C-ROS ALONG THE EXCURRENT DUCT IN HUMANS
C. Légaré, N. Verville, R. Sullivan

P1/2-013 SPERM PROGRESSION THROUGH THE EPIDIDYMIS (EPI) IS REGULATED NOT ONLY BY NERVE
FIBRES BUT ALSO BY HORMONAL FACTORS, AS OXYTOCIN (OT)

P1/2-014 DIETARY LIPIDS, EPIDIDYMIS AND ACTIVITY OF GAMMA-GLUTAMYLTRANSPEPTIDASE
M.B. Medina, A.R. Eynard, M. Valentich

P1/2-015 PROCESSING AND LOCALIZATION OF THE D AND E FORMS OF RAT CRISP-1
K.P. Roberts, K.E. Ensurd, D.W. Hamilton
P1/2-016 EVIDENCE THAT HEPARAN SULPHATE IS A NUCLEAR DECONDENSING AGENT OF HUMAN SPERMATOZOA IN VIVO
M. Romanato, G. Bertolesi, J.C. Calvo, L.P. Calvo

P1/2-017 FERTILIZATION POTENTIAL OF SPERMATOZOA IN BLOCKED EPIDIDYMIDES
R. Schoysman, P. Van der Zwalm

P1/2-018 IDENTIFICATION AND SEQUENCE ANALYSIS OF A GENOME FRAGMENT ENCODING A mE-RABP RELATED HUMAN ORTHOLOGUE, hE-RABP
K. Suzuki, Y. Araki, J.J. Lareyre, R.J. Matusik, M.C. Orgebin-Crist

P1/2-019 CUBILIN AND LRP-2 EXPRESSION IN RAT EPIDIDYMIS
O. Van Praet, J. Rattenbury, C.R. Morales, C. Knaak, W.S. Argraves, S. Lefrançois

P1/2-020 EXPRESSION OF MOUSE EPIDIDYMAL SPAM1 (PH-20), A SECRETORY PROTEIN, IN VIVO AND IN VITRO
H. Zhang, P.A. Martin-DeLeon

Spermatozoa 1

P1/2-021 ROLE OF ZINC IN BUFFALO SPERM CAPACITATION AND ACROSOME REACTION
G.S. Bilaspuri, B.K. Babbar, G.K. Sangha

P1/2-022 ACROSOMAL PROTEINASE ACTIVITY OF HUMAN TESTICULAR SPERM AND EPIDIDYMAL SPERM
W.-J. Zhu, W.-B. Liang, J. Qing-Liu

P1/2-023 CHOLESTEROL REDISTRIBUTION AND EFFLUX IN CAPACITATING SPERM CELLS
F.M. Flesch, I.F.H.M. Brouwers, P.F.E.M. Nievelstein, B.M. Gadella

P1/2-024 RAT CALTRIN (CALCIUM TRANSPORT INHIBITOR) INHIBITS ACTIVATION AND ACTIVITY OF HUMAN SPERM ACROSOMAL PROTEASES
J. Biancotti, L. Furlong, M. Novella, C. Coronel, M. Vazquez-Levin

P1/2-025 IMPORTANCE OF CALCIUM DURING BOAR SPERM CAPACITATION
C. Dubé, S. Tardif, J.L. Bailey

P1/2-026 EXPRESSION OF P450 AROMATASE TRANSCRIPTS IN EJACULATED HUMAN SPERMATOZOA
I. Galeraud-Denis, S. Lambard, S. Carreau

P1/2-027 REGULATION OF AROMATASE GENE EXPRESSION IN ADULT MALE RAT GERM CELLS: EFFECTS OF TGFβ, TNFα AND TESTOSTERONE
S. Bourguiba, M. Benahmed, S. Carreau

P1/2-028 CALCIUM REQUIREMENTS FOR THE MAINTENANCE OF HUMAN SPERM FUNCTION IN VITRO

P1/2-029 DIFFERENTIAL EXPRESSION OF PHOSPHOTYROSINE EPITOPES IN MAMMALIAN SPERMATOZOA FROM DIFFERENT REGIONS OF THE CAUDA EPIDIDYMIS
I.A. Brewis, S.E. Boussouf, N.M. Jenkins, H.D.M. Moore
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P1/2-030  MULTIPLE TYPES OF PHOSPHODIESTERASE ISOFORMS IN HUMAN SPERMATOZOA: PARTIAL CHARACTERIZATION
L. Lefèvre, E. de Lamirande, S.D. Rybalkin, J.A. Beavo, C. Gagnon

P1/2-031  ROLE OF TYROSINE KINASE (PTK) AND cAMP-DEPENDENT KINASE (PKA) CROSS-TALK IN THE REGULATION OF HUMAN SPERM MOTILITY
M. Baijpai, G.F. Doncel

P1/2-032  IDENTIFICATION OF SRC FAMILY TYROSINE KINASES IN BULL GERM CELLS BY RT-PCR
C. Lalancette, P. Leclerc

P1/2-033  cAMP CONCENTRATION INCREASES DURING ACTIVATION OF MOTILITY IN QUIESCENT CAUDAL EPIDIDYMAL SPERM FROM THE RAT
M. Wade, R.C. Jones, R.N. Murdoch, R.J. Aitken

P1/2-034  INVOLVEMENT OF STORE OPERATED CHANNELS IN THE SUSTAINED COMPONENT OF THE PROGESTERONE-INDUCED [CA^{2+}] SIGNAL IN HUMAN SPERMATOZOA
E.L. Punt, C.L.R. Barratt, S.J. Publicover

P1/2-035  FACTORS REGULATING TYROSINE PHOSPHORYLATION OF MURINE SPERMATOZOA
H. Ecroyd, R.J. Aitken, R.C. Jones

P1/2-036  REGULATION OF HUMAN SPERM CAPACITATION AND PROTEIN TYROSINE PHOSPHORYLATION BY CA^{2+} -ATPase
V. Dorval, P. Leclerc

P1/2-037  CASPASES ARE ASSOCIATED WITH HUMAN SPERMATOGENESIS AND APOPTOSIS IN EJACULATED SPERMATOZOA
U. Paasch, S. Grunewald, K. Wündrich, H. Glander

P1/2-038  INCIDENCE OF TAIL STRUCTURE DISTORTIONS ASSOCIATED WITH DYSPLASIA OF THE FIBROUS SHEATH IN HUMAN SPERMATOZOA
V. Rawe, V. Galaverna, S.B. Olmedo, A. Acosta, H. Chemes

P1/2-039  MULTIPLE POINT MUTATIONS IN THE MURINE SPAM1 GENE ARE ASSOCIATED WITH SPERM DYSFUNCTION IN MICE BEARING THE RB(6.16)24LUB OR RB(6.15)1ALD TRANSLOCATION
Y. Zheng, X.N. Deng, P.A. Martin-DeLeon

P1/2-040  EXPRESSION OF GM-CSF RECEPTORS IN BOVINE MALE GERM CELLS
L.T. Vilanova, M.C. Rauch, M. Brito, I.I. Concha

P1/2-041  PRESENCE AND DISTRIBUTION OF ACTIN ANCHORING PROTEINS IN BULL SPERMATOZOA DURING AND FOLLOWING EPIDIDYMAL TRANSIT
M.E. Carbajal, R.M. Pelletier, S.R. Yoon, M.L. Vitale

P1/2-042  APOPTOSIS: TRANSLOCATION OF PHOSPHATIDYLSERINE AND NICKED DNA IN BULL SPERM, DETECTED BY FLOW CYTOMETRY, AND THEIR RELATIONSHIPS WITH FERTILITY
M. Anzar, L. He, M.M. Buhr, T. Kroetsch, K.P. Pauls
Sperm-Oocyte Interaction

P1/2-043 CAPACITATED AND CHEMOTACTIC RABBIT SPERMATOZOA APPEAR TO BEShortly AVAILABLE AROUND OVULATION
L. Giojalas, G. Fabro, M. Eisenbach, R. Rovasio

P1/2-044 DIRECTIONALITY-BASED ASSAY FOR SPERM CHEMOTAXIS, INDEPENDENT OF CHEMOKINESIS AND THE SWIMMING PATTERN
L. Giojalas, M. Eisenbach, S. Civalero, A. Frenkel, A. Chernogorova, R. Rovasio

P1/2-045 ANALYSIS OF THE IMPACT OF CAFFEINE ON MEMBRANE INTEGRITY, REDOX RATIO AND GST IN HUMAN EJACULATED SPERM : EFFECTIVENESS OF ANTIOXIDANTS
M. Arabi, R.J.K. Anand, U. Kanwar

P1/2-046 EFFECTS 17β-PROGESTERONE, 17β-ESTRADIOL AND RU486 ON HUMAN SPERM FUSION WITH OOCYTES. COMPARISON AND INTERERENCE WITH THE EFFECT OF PROGESTERONE
F. Francavilla, R. Romano, C. Pandolfi, R. Santucci, B. Macerola, S. Franvavilla

P1/2-047 IDENTIFICATION OF E, P, AND N CADHERINS IN HUMAN SPERMATOZOA
A. Lasserre, C. Marin-Brigilier, C. Guaragna, G. Rey Valzacchi, M. Vazquez-Levin

P1/2-048 ROLE FOR SPERAD?

P1/2-049 IN VITRO FERTILIZING CHARACTERISTICS OF BOVINE SPERM WITH ABNORMAL MORPHOLOGY
J.C. Thundathil, A.D. Barth, R.J. Mapleton

P1/2-050 CHARACTERISATION OF HUMAN SPERM ANTIGENS EXPOSED AFTER THE ACROSOME REACTION THAT MAY PARTICIPATE IN GEMETE FUSION PROCESSES
N. Al-Eisa, R.O. Ojoo, H.D.M. Moore

P1/2-051 FINE MAPPING AND CHARACTERIZATION OF THE Stop1d (SPERM-t COMPLEX-OOCYTE PENETRATION 1-DISTAL) LOCUS IN THE t HAPLOTYPE REGION OF Mus musculus
A.A. Redkar, L. Hui, P. Olds-Clarke, S.H. Pilder

P1/2-052 ANALYSIS OF THE ROLE OF CARBOHYDRATES AND DISULFIDE BONDS IN THE BIOLOGICAL ACTIVITY OF EPIDIDYMAL PROTEIN DE
D.A. Ellerman, M.M. Morgenfeld, V.G. Da Ros, D. Busso, D.J. Cohen, P.S. Cuasnicu

P1/2-053 PT32, A POTENTIAL CANDIDATE FOR A SPERM BORNE EGG ACTIVATING FACTOR
A.T.H. Wu, P. Sutovsky, T. Dominko, G. Schatten, J. Gong, R. Oko

P1/2-054 RUFLING OF THE ACROSOME OF HUMAN SPERM

P1/2-055 MODULATION OF HUMAN SPERM CAPACITATION BY ENDOMETRIAL CELLS
J. Laflamme, A. Akoum, P. Leclerc
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Androgens

P1/2-056 EFFECTS OF TESTOSTERONE ON BEHAVIOUR AND MOOD IN EUGONADAL AND HYPOGONADAL MEN
D.B. O'Connor, J. Archer, F.C.W. Wu

P1/2-057 ANDROGEN FUNCTION OF ADRENALS AND TESTES IN PATIENTS WITH HYPERGONADOTROPHIC HYPOGONADISM
M. Koryakin, A.S. Akopyan

P1/2-058 EFFECT OF FOOD ON THE ORAL BIOAVAILABILITY OF A NEW ANDRIOL® FORMULATION
W.M. Bagchus, P.G. Schnabel, F. Maris, N.S. Houwing

P1/2-059 DOSE PROPORTIONALITY OF A NEW ANDRIOL® FORMULATION
N.S. Houwing, F. Maris, P.G. Schnabel, W.M. Bagchus

P1/2-060 THE U.S. ANTIDOPING (USDA) RESEARCH SUMMIT SUMMARY
J.L. Fourcroy

Semen Analysis

P1/2-061 L-CARNITINE AND FERTILITY IN RAMS
G. El-Amrawi, W.M.B. Noseir

P1/2-062 NO DECLINE IN SPERM QUALITY IN A COMMUNITY BASED STUDY OF MEN UNDERGOING VASECTOMY WITHIN 20 YEARS
A. Jungwirth, E. Rovan, K. Fink, N. Schmeller

P1/2-063 PREPARATION OF A MORPHOMETRIC STANDARD FOR HUMAN SPERM
S. Kaneko, H. Ishiakwa, K. Miyaji, T. Iwamoto, K. Baba, K. Tanabe

P1/2-064 STunted Tail Sperm Defect: An Ultrastructural Study of an Atypical Case

P1/2-065 EXTERNAL QUALITY ASSESSMENT OF HUMAN SPERM MORPHOLOGY DURING A WORKSHOP INVOLVING 62 PARTICIPANTS
F. Eustache, J. Auger

P1/2-066 LOWER PERCENTAGE OF SPERM WITH NORMAL MORPHOLOGY (NM) USING STRICT CRITERIA IS NOT ASSOCIATED WITH LOWER PREGNANCY RATES (PRs) FOLLOWING INTRAUTERINE INSEMINATION (IUI)
A. Bollendorf, J.H. Check, D. Katsoff

P1/2-067 EVALUATION OF THE HYPO-OSMOTIC SWELLING TEST IN RELATION WITH SPERM CONCENTRATION AND MOTILITY
P1/2-068  ANXIETY AND CHANGES IN SPERMIOGRAMM PARAMETERS
A.S. Gozen, T.G. Delci, B. Alagol, S. Molla

P1/2-069  EVALUATION OF TISSUE FACTOR AND INTERLEUKIN-6 LEVEL IN SEMINAL PLASMA
S. Ohta, H. Wada, T. Nobori, H. Fuse

P1/2-070  SEMINAL MICROFLORA IN INFERTILE AND CHRONIC PROSTATITIS PATIENTS
R. Mändar, M. Punab, K. Lõivukene, K. Kermes

P1/2-071  EFFECTS OF BACTERIA ON SEMEN PARAMETERS
T.R. Weerasooriya, L. Fernando, P.M. Rodrigo, A. Wattage

P1/2-072  FLOW CYTOMETRIC METHODS TO MEASURE HUMAN SPERM CONCENTRATION
F. Eustache, P. Joannet, J. Auger

P1/2-073  CHARACTERIZATION FROM SPERM CAPACITATION IN Agouti paca AND Agouti taczanowskii

P1/2-074  SPERM CHROMATIN STRUCTURE ASSAY (SCSA) SHOWS CORRELATION WITH SPERM MORPHOMETRY

P1/2-075  A NEW PROCEDURE FOR DETECTING DNA FRAGMENTATION IN HUMAN SPERMATOZOA USING THE TUNEL METHOD
T. Nishida, S. Kaneko, M. Yoshiiike, S. Nozawa, K. Tanabe, T. Iwamoto

P1/2-076  LABORATORY METHODS FOR THE DIAGNOSIS OF ASTHENozoospermia

P1/2-077  EFFECT OF THE SEMEN COLLECTION PLACE ON THE SEMEN QUALITY

P1/2-078  SEMEN QUALITY OF JAPANESE GENERAL POPULATION

P1/2-079  REPRODUCTIVE STATUS OF MALE GIANT PANDAS

P1/2-080  INFLUENCE OF H+-CONCENTRATION ON THE VIABILITY OF A23187-TREATED HUMAN SPERMATOZOA
J. Skrzypek, F. Schwarzinger, W. Krause

P1/2-081  FUNCTIONAL TESTS AS THE MARKERS OF Fertilizing CAPACITY OF SPERMATOZOA
N. Lalic, S. Micic, N. Bojanic, N. Majkic-Singh
Male Fertility and Infertility 1

P1/2-082 THE HUMAN SPERM HEAD, A KEY FOR SUCCESSFUL FERTILISATION
A.A. El-Ghobashy, C.R. West, D.I. Lewis-Jones

P1/2-083 THE EFFECT OF SPERM WITH HYPO-OSMOTIC SWELLING TEST (HOST)SCORES IN THE GREY ZONE AND SUBSEQUENT TREATMENT WITH CHYMOTRYPSIN ON IN VITRO FERTILIZATION (IVF) OUTCOME
D. Kiefer, J.H. Check, C. Wilson, M.L. Check, J. Locuniak

P1/2-084 THE RELATIVE DISCREPANCY BETWEEN VIABILITY AND HYPO-OSMOTIC SWELLING TEST (HOST) SCORES IS NOT RELATED TO IN VITRO FERTILIZATION (IVF) OUTCOME.
J.H. Check, D. Kiefer, M.L. Check, C. Wilson, J.K. Choe

P1/2-085 SPERM NUCLEAR DECONDENSATION ASSESSED BY CONFOCAL MICROSCOPY AND FLOW CYTOMETRY IN HUMAN INFERTILITY

P1/2-086 ROLE OF THE SPERM CHROMATIN STRUCTURE ASSAY (SCSA) IN THE HUMAN INFERTILITY CLINIC
D.P. Evenson, L. Jost, K. Larson, J. Brannian, K. Hansen, D. Kreger

P1/2-087 COMPARISON OF SEMEN CYTOLOGY & DIAGNOSTIC TESTIS BIOPSY IN DIAGNOSIS OF MALE INFERTILITY
Y-F. Huang, N-Q. LU

P1/2-088 SPERM CHROMATIN STRUCTURE ASSAY DOES NOT PREDICT EARLY EMBRYO DEVELOPMENT IN VITRO
J. Kossakowski, R. Thus, J. Sapoñikov, J. Catt

P1/2-089 ULTRASTRUCTURAL ANALYSIS OF CHROMATIN DEFECTS IN TESTICULAR SPERMATIDS OF AZOOSPERMIC MEN SUBMITTED TO TESE-ICSI
S. Francavilla, M.A. Bianco, G. Cordeschi, P. D'Abrizio, C. De Stefano, F. Francavilla

P1/2-090 MOLECULAR CONTROL OF FERTILIZING POTENTIAL OF MALE GAMETE IN AZOOSPERMIA: A STUDY OF GENES ENCODING RBM, DAZ AND TSPY

P1/2-091 SERTOLI CELL FUNCTION IN INFERTILE PATIENTS WITH AND WITHOUT Y CHROMOSOME MICRODELETIONS
A. Ferlin, A. Bettella, E. Moro, A. Garolla, M. Merico, M. Rossato, C. Foresta

P1/2-092 SCREENING FOR THE PRESENCE OF THE AZF (AZOOSPERMIA FACTOR) -CANDIDATE GENES IN IDIOPATHIC AZOOSPERMIA AND OLIGOZOOSPERMIA
E. Koh, A. Mizokami, M. Namiki

P1/2-093 FAMILIAL AZOOSPERMIA AND OLIGOSPERMIA
S. Segal, Z. Palti, A. Rosenman, I. Tur-Kaspa, S. Meltzer, A. Shultz, O. Gemer

P1/2-094 Y CHROMOSOME HAPLOGROUPS AND MALE REPRODUCTIVE FUNCTION
M. Punab, M. Adojaan, S. Rootsi
Saturday, June 16

P1/2-095 CFTR GENE MUTATIONS IN INFERTILE MEN
J.K. Wolski, A. Sobczynska-Tomaszewska, T. Mazurczak, J. Bal, K. Kozioł, P. Lewandowski

P1/2-096 ASSOCIATION BETWEEN THE HLA-DR AND DQ ALLELES AND MALE INFERTILITY
A.E. Alenany, I.D. Cooke, H.D. Moore

P1/2-097 UTILITY OF INVESTIGATION OF ALLELES OF CFTR GENE IN INFERTILE MEN

P1/2-098 IS SCREENING FOR Y CHROMOSOME MICRODELETION IN PATIENTS UNDERGOING INTRACYTOPLASMIC SPERM INJECTION ALWAYS NECESSARY?
E. Szerman, G. Plessis, J.M. Lévique, F. Comoz, A. Sauvalle, M.L. Kottler

P1/2-099 MICRODELETIONS OF THE CA2+ CHANNEL WITHIN TESTICULAR TISSUE AND SPERM. SHOULD THESE MEN HAVE ICSI?
J.L. Marmar, C. Millan, I.R. Hurley, S. Benoff

P1/2-100 INFLUENCE OF STRESS ON SPERM PARAMETERS OF INFERTILE MEN
S. Micic, N. Lalic, N. Bojanic, O. Durutovic, I. Vukovic

P1/2-101 THE AGING EFFECT OF MEN ON SEXUAL BEHAVIOR AND SEMINAL PROFILES: FACT OR FICTION?
P.M. Zavos, P. Aslanis, K. Kaskar, J.R. Correa, P.N. Zarmakoupis-Zavos

P1/2-102 FAILURE OF PIASx Expression in Human Testes of Men With Non-Obstructive Azoospermia: Potential Etiologic Factor in Defective Human spermogenesis
P.T. Chan, A. Mielenik, P.L. Morris, P.N. Schlegel

P1/2-103 MALE INFERTILITY: IS AFRICA DIFFERENT?
M.S.Bornman, C.F. Yssel, M. Roux

P1/2-104 INHIBITION OF IN VITRO FERTILIZATION IN THE HAMSTER BY ANTIBODIES RAISED AGAINST THE RAT SPERM PROTEIN SP22

P1/2-105 RECURRENT MISCARRIAGE: COULD THE MALE PARTNER BE RESPONSIBLE?
A.J. Campbell, P. Bishton, E.H. Gordon, D.S. Irvine

Varicocele

P1/2-106 VARICOCELECTOMY REDUCES REACTIVE OXYGEN SPECIES LEVELS AND INCREASES ANTIOXIDANT ACTIVITY OF SEMINAL PLASMA OF INFERTILE MEN WITH VARICOCELE
M.T. Anis, T. Mostafa, A. Al-Nashar, H. Emam, I. Osman

P1/2-107 VARICOCELE ELEVATES REACTIVE OXYGEN SPECIES LEVELS AND REDUCES ANTIOXIDANT ACTIVITY OF INTERNAL SPERMATIC VEIN BLOOD OF INFERTILE MEN WITH VARICOCELE
M.T. Anis, T. Mostafa, A. El-Nashar, H. Imam, I.A. Othman

P1/2-108 SOMATIC ACE ACTIVITY IN LEFT SPERMATIC VEIN SAMPLES OF PATIENTS WITH LEFT VARICOCELE AND ITS CORRELATION TO THE SPERMATOLOGIC PARAMETERS
R. Asci, S. Sarikaya, A. Bedir, R. Buyukalpelli, A.F. Yilmaz
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P1/2-109 PROINFLAMMATORY SEMINAL CYTOKINES AND SEMEN PARAMETERS IN VARICOCELE
E. Cardoso, G. Noya, A. Almos, J. Santoianni, L.N. Contreras, A.L. Arregger

P1/2-110 CARNITINE AND ACETILCARNITINE TREATMENT IMPROVES SPERM MOTILITY AND VIABILITY
AFTER VARICOCELECTOMY
M. De Rosa, S. Zarrilli, B. Amalfi, L. Paesano, G. Lombardi

P1/2-111 ULTRASTRUCTURAL CHANGES OF THE SPERMATIC VEINS IN VARICOCELE
A.A. El-Kamshoushi, W. Abdallah, Y. Kosba, S. Helal

P1/2-112 VALUE OF TESTICULAR BIOPSY DUE TO THE MICROSURGICAL CORRECTION OF BILATERAL
VARICOCELE (VC) ASSOCIATED WITH AZOOSPERMIA (AZO)

P1/2-113 THE GONANDROTROPIN RELEASING HORMONE (GnRH) STIMULATION TEST MAY PREDICT
PREGNANCY OUTCOME AFTER MICROSURGICAL VARICOCELECTOMY
M.A. Fischer, K.M. Khamel, K. Jarvi, A. Zini

P1/2-114 THE INFLUENCE OF UNILATERAL AND BILATERAL LAPAROSCOPIC VARICOCELECTOMY
ON PARAMETERS OF FERTILITY IN 290 MEN
J.Huk, M. Fryczkowski

P1/2-115 TOTAL ANTIOXIDANT CAPACITY IN SEMINAL FLUID OF VARICOCELE PATIENTS:
CORRELATION WITH SPERM MOTILITY
A. Mancini, E. Meucci, D. Milardi, A. Bianchi, E. Giacchi, L. De Marinis, L. Jensen

P1/2-116 SCROTAL TEMPERATURE AND VARICOCELECTOMY : VARICOCELE OR VARICOCELES?
R. Mieusset, E. Huyghe, M. Daudin, C. Tollon, F. Pontonnier, L. Bujan

P1/2-117 TOTAL ANTIOXIDANT CAPACITY IN SEMINAL FLUID OF VARICOCELE PATIENTS:
CORRELATION WITH HORMONE PATTERN
D. Milardi, A. Mancini, E. Meucci, A. Bianchi, E. Giacchi, L. Jensen, L. De Marinis

P1/2-118 VARICOCELE TREATMENT: EVOLUTION IN SCLEROSING TECHNIQUES
S. Minucci, G. Mazzoni, V. Gentile

P1/2-119 TESTICULAR VOLUME IN INFERTILE VARICOCELE PATIENTS WITH NORMAL
AND ABNORMAL FOLLICLE-STIMULATING HORMONE
P. Moreira de Góes, F.F. Pasqualotto, A.M. Lucon, J. Hallak, S. Arap

P1/2-120 SEMINAL REACTIVE OXYGEN SPECIES (ROS) IN VARICOCELE PATIENTS: PRELIMINARY RESULTS
DEMONSTRATE TREND OF INCREASE ROS LEVELS WITH VARICOCELE GRADE, NOT TESTIS SIZE

P1/2-121 EFFICACY OF SUBINGUINAL VARICOCELECTOMY ON SEMEN PARAMETERS AND PREGNANCY
IN MEN WITH SEVERE OLIGOZOOSPERMIA
K. Ogura, J. Watanabe, K. Okubo, Y. Arai

P1/2-122 VARICOCELE REPAIR IMPROVES SEMEN PARAMETERS IN AZOOSPERMIC MEN
WITH TESTICULAR FAILURE IRRESPECTIVE TESTICULAR HISTOLOGY
F.F. Pasqualotto, A.M. Lucon, J. Hallak, L.B. Saldanha, P.M. Góes, J.R. Colombo, S. Arap
Saturday, June 16

P1/2-123 HORMONE LEVELS AND TESTICULAR VOLUME IN FERTILE MEN WITH VARICOCELES, INFERTILE MEN WITH VARICOCELES, AND FERTILE CONTROLS WITHOUT VARICOCELES
F.F. Pasqualotto, P.M. Góes, A.M. Lucon, J. Hallak, B.C.P. Jeng, S. Arap

P1/2-124 INCIDENCE OF VARICOCELE IN CHILDREN AND ADOLESCENTS
K.H. Rha, B.H. Kim, S.W. Han, M.S. Lee

P1/2-125 MICROVASCULAR TESTICULAR BLOOD FLOW AS EVALUATED BY LASER DOPPLER FLOWMETRY AFTER THE SURGICAL INDUCTION OF VARICOCELE
N. Salama, A. Bergh, J.E. Damber

P1/2-126 THE CHANGES IN TESTICULAR VASCULAR PERMEABILITY DURING PROGRESSION OF THE EXPERIMENTAL VARICOCELE
N. Salama, A. Bergh, J.E. Damber

P1/2-127 THE INCIDENCE, SPECIFICITY AND SENSITIVITY OF CLINICAL EXAM FOR THE DIAGNOSIS OF VARICOCELE COMPARED TO DOPPLER ULTRASOUND
O. Shah, S. Telegraf, A.R. McCullough

P1/2-128 SURGICAL TREATMENT OF VARICOCELE – CONTROVERSIAL PROBLEM
A LONG-TERM FOLLOW-UP STUDY
G.A. Szymczyński

P1/2-129 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NITRIC OXIDE SYNTHASE (NOS) ISOENZYMES IN HUMAN VARICOCELE AND EXPERIMENTAL RAT VARICOCELE
J.K. Tan, K. Ameer, E.A. Ling

P1/2-130 MICROMEASUREMENTS OF MICROCIRCULATION PERFUSION AND THERMAL PARAMETERS IN HUMAN TESTES DURING MICROSURGERY OF VARICOCELE
J. Tritto

P1/2-131 DETERIORISATION OF SPERM PARAMETERS IN MEN WITH UNTREATED VARICOCELE
I. Vukovic, N. Bojanic, N. Lalic, S. Micic

Contraception

P1/2-132 THE EFFECT OF PROGESTINS ON SERUM TESTOSTERONE, AGGRESSION AND SEMEN PRODUCTION IN GERENUK (LITOCRANIUS WALLERI WALLERI)
L. Penfold, E. Plotka, S.B. Citino

P1/2-133 ACHIEVING AZOOSPERMIA BY INJECTIONS OF TESTOSTERONE UNDECANOATE ONLY OR COMBINED WITH DEPOT MEDROXYPROGESTERONE ACETATE IN INDONESIAN MEN (JAKARTA CENTRE STUDY)
N. Moeloek, D.A. Pujianto, R. Agustin

P1/2-134 HUMAN SPERMATOZOA MEMBRANE ASSOCIATED CHANGES IN PRESENCE OF RISUG-A NEW INJECTABLE MALE CONTRACEPTIVE
K. Chaudhury, A.K. Bhattacharyya, K. Guha
**Poster Session 1/2**

**Please note:** Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

P1/2-135 **EFFECTS OF A LONG-ACTING PROGESTAGEN IMPLANT (NORPLANT II) WITH ANDROGEN PREPARATIONS IN THE SUPPRESSION OF SPERMATOGENESIS IN NORMAL MEN**

P1/2-136 **ANTIFERTILITY EFFECT OF ACTIVE IMMUNIZATION WITH THE C-TERMINAL 67-94 REGION (R-28) OF HUMAN SEMINAL PLASMAINHIBIN**

P1/2-137 **SERUM GONADOTROPINS & GERM CELL DEVELOPMENT WITH TESTOSTERONE ± PROGESTIN CONTRACEPTION**

P1/2-138 **CURCUMIN, A NATURAL HERB WITH ANTI-HIV ACTIVITY INHIBITS HUMAN SPERM MOTILITY**
M. Rajasekaran, T. Rithaporn, M. Monga

P1/2-139 **EFFECT OF LEAF EXTRACT OF NEEM (AZADIRACHTA INDICA) ON THE REPRODUCTIVE ORGANS OF MALE MOUSE**
R.K. Mishra, S.K. Singh

P1/2-140 **7α-METHYL-19-NORTESTOSTERONE (MENT™) IMPLANTS FOR MALE CONTRACEPTION: A DOSE-FINDING STUDY**

**Sexually Transmitted Diseases**

P1/2-141 **FREQUENCY OF HERPES SIMPLEX VIRUS, CYTOMEGALOVIRUS AND HUMAN PAPILLOMAVIRUS DNA IN SEMEN**
O. Aynaud, J.D. Poveda

P1/2-142 **EFFECTIVENESS OF SPERM WASHING TO RECOVER SPERMATOZOA WITHOUT HIV AND HCV GENOMES DETECTION IN HIV INFECTED MEN**

P1/2-143 **HIV SEROPOSITIVITY AND MALE INFERTILITY: A CLINICAL DILEMMA**
M.S. Bornman, G. Schulenburg

P1/2-144 **MYCOPLASMA AND CHLAMYDIA ETIOGENIC FACTORS OF CONYUGAL INFERTILITY**

P1/2-145 **NONSPECIFIC GENITAL INFECTIONS DIAGNOSED BY FRESH CYTOLOGICAL ANALYSIS**
B. Ramos-González, G. Gallegos-Avila

P1/2-146 **EFFICIENCY OF ARTIFICIAL INSEMINATION IN GIANT PANDAS AT THE WOLONG BREEDING CENTER**
Spermatozoa2

P3/4-001  EXPRESSION OF CFTR IN EJACULATED SPERMATOZOA

P3/4-002  SURFACE MAPPING OF OVIDUCTIN OVER THE PLASMA MEMBRANE OF GOLDEN HAMSTER
SPERMATOZOA DURING IN VITRO CAPACITATION AND ACROSOME REACTION
F.W.K. Kan, P. Esperanzate

P3/4-003  COMPOSITION AND DEVELOPMENTAL EXPRESSION OF PROTEINS CONSTITUTING THE SPERM
HEAD CYTOSKELETON OF THE PLAINS RAT
D. Idriss, W.G. Breed, R.J. Oko

P3/4-004  ISOLATION AND PERINUCLEAR IMMUNOLOCALIZATION OF SOMATIC HISTONES H2B
AND H3 IN BULL SPERM HEADS
P.R. Tovich, M. Oda, Y. Yu, R. Oko

P3/4-005  EXPRESSION OF P2Y PURINERGIC RECEPTOR IN HUMAN SPERM
M. Rossato, E. Moro, P. Marin, A. Rossi, C. Galeazzi, C. Foresta

P3/4-006  ISOLATION AND CHARACTERIZATION OF CAPACITATING FACTOR (GLYCOSAMINOGLYCANS)
FROM THE BOVINE FOLLICULAR FLUID
I. Therien, M-H. Crete, P. Manjunath

P3/4-007  A COMPARATIVE STUDY OF THREE BUFFER SYSTEMS OF YOLK-FREE HUMAN SEMEN
CRYOPROTECTIVE MEDIA
Y. Kang-Shou, W-J. Zhu

P3/4-008  ENHANCED CAT SPERM CRYOPRESERVATION
B.S. Pukazhenthli, D.E. Wildt, J.G. Howard

P3/4-009  THE EFFECT OF PROSTAGLANDINS ON CHILDBIRTHING FUNCTION OF WOMEN INSEMINATED
WITH FROZEN- THAWED SPERM
V. Avagyan, S.B. Grigoryan, V.K. Nazaryan

P3/4-010  THE MEDIUM FOR LOW-TEMPERATURE CONSERVATION OF HUMAN SPERM
V. Avagyan, S.B. Grigoryan, V.K. Nazaryan

P3/4-011  INFLUENCE OF BOVINE OVIDUCT EPITHELIAL CELL APICAL PLASMA MEMBRANE
ON CRYOPRESERVED SPERMATOZOA
M. Boilard, M.A. Sirard

P3/4-012  LONG-TERM OUTCOMES OF ELECTIVE SPERM CRYOSTORAGE

P3/4-013  EFFECTS OF BICARBONATE ON HEAD-TO-HEAD AGGLUTINATION IN BOAR SPERMATOZOA
H. Harayama

P3/4-014  COMET ASSAY RELIABILITY IN CRYOPRESERVED HUMAN SPERM CELLS.
Please note: Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

P3/4-015  THE ROLE OF A FUCOSE-BINDING PROTEIN IN SPERM BINDING TO BOVINE OVIDUCTAL EPITHELIUM.
T.M. Gwathmey, G.G. Ignotz, S.S. Suarez

P3/4-016  REGULATION OF HUMAN SPERM VOLUME
C-H. Yeung, T.G. Cooper

P3/4-017  EFFECTS OF EXTRACELLULAR pH AND BUFFERING AGENTS ON MOTILITY OF FROZEN/THAWED BOVINE SPERMATOZOA.
S. Guay, M.A. Sirard, P. Leclerc

P3/4-018  THE HYPO-OSMOTIC SWELLING (HOS) CLASSIFICATION AND GRADING SYSTEM OF HUMAN SPERM
F.N. Wang, B.Y. Suh

P3/4-019  ANALYSIS OF ASIAN mtDNA HAPLOGROUPS IN LOW AND NORMAL SPERM MOTILITY
Sudjarwo, A. Hinting, H. Sudoyo, S. Marzuki

P3/4-020  PLATELET-ACTIVATING FACTOR ACTIVITY LEVELS (LIGAND AND RECEPTOR TRANSCRIPT) CONTENT IN SPERMATOZOA: MOTILE VERSUS NONMOTILE
E.T. Purnell, W.E. Roudebush

P3/4-021  TREATING BULL SPERM WITH CHOLESTEROL LOADED CYCLODEXTRIN IMPROVES CRYO SURVIVAL
P.H. Purdy, J.K. Graham

P3/4-022  LOCALIZATION OF SP22 ON HUMAN SPERM OF DIFFERING QUALITY

P3/4-023  INFLUENCE OF ANGIOTENSINS ON HUMAN SPERM MOTILITY
E. Aleandre-Lafont, F.M. Köhn, W.B. Schill, C. Müller

P3/4-024  PURIFICATION OF HUMAN SEMINAL PHOSPHOLIPID-BINDING PROTEINS CLOSELY RELATED TO THE MAJOR PROTEINS OF BOVINE SEMINAL PLASMA
M. Menard, P. Manjunath

Spermatogenesis 1

P3/4-025  TWO NOVEL METHODS FOR GERM CELL TRANSPLANTATION (GCT)
N. Sofikitis, Y. Yamamoto, I. Miyagawa

P3/4-026  ISOLATION AND CULTURE OF ENRICHED POPULATION OF TYPE A-SPERMATOGONIA FROM PREPUBERTAL BULL TESTES
F. Izadyar, L.B. Creemers, K. den Ouden, D.G. de Rooij

P3/4-027  CHARACTERIZATION OF SPERMATOGONIAL TYPES IN MOUSE SPERMATOGENESIS UTILIZING SEMI-THIN SECTIONS OF PERFUSED TISSUE
H. Chiarini-Garcia, L.D. Russell
Sunday, June 17  

Poster Session 3/4

P3/4-028  
THE SPATIAL ORGANIZATION OF SPERMATOGENESIS IS REGULATED BY THE STAGE-RELATED TOPOGRAPHY OF TYPE A SPERMATOGONIA IN THE MOUSE  

P3/4-029  
TELOMerase IMMORTALIZED SPERMATOGONIAL CELL LINES  
L. Feng, Y. Chen, R. Reijo Pera, M-C. Hofmann, M. Dym

P3/4-030  
QUANTIFICATION OF HUMAN TELOMerase REVERSE TRANSCRIPTASE MRNA EXPRESSION IN TESTICULAR TISSUE OF INFERTILE PATIENTS  
M. Schrader, M. Müller, R. Heicappell, B. Straub, K. Miller

P3/4-031  
THE REGULATION OF TELOMerase ACTIVITY AND TELOMERE LENGTH IN THE STERILE RAT'S TESTIS  
I. Oh' hara, T.T. Tomura

P3/4-032  
DNA DAMAGES IN SUBSETS OF HUMAN SPERMATOZOA AT DIFFERENT STAGES OF MATURATION  

P3/4-033  
ALTERED CREM LOCALIZATION IN SPERMATOCYTIC CELLS IN TESTOSTERONE (T)-TREATED SPINAL CORD INJURED (SCI) RATS  
H.S. Huang, R. Anesetti, C.A. Molina, B. West, S.L. Wang, J.E. Ottenweller, L.M. Pogach

P3/4-034  
ISOLATION AND CHARACTERIZATION OF HAPLOID GERM CELL SPECIFIC NOVEL CDNA; TESTIS-SPECIFIC HOMOLOGUE OF SUCCINYL COA: 3-OXO ACID COA TRANSFERASE (SCOT-T)  

P3/4-035  
RATE OF HOMOLOGOUS CHROMOSOME PAIRING IN SPERMATOCYTES MAY PREDICT COMPLETION OF THE SPERMATOGENESIS PROCESS IN TESTES OF AZOOSPERMIC MEN  

P3/4-036  
DNA DAMAGE IN GERM CELLS IS NOT FULLY REPAIRED DURING SPERMATOGENESIS  
S.A. King, P.T.K. Saunders, D.S. Irvine

Testis 1

P3/4-037  
cDNA CLONING AND SEQUENCE ANALYSIS OF PROSTAGLANDIN D SYNTHASE IN HUMAN TESTIS  
J.C. Lu, Y.F. Huang, X.R. Zhang

P3/4-038  
CONSTRUCTION AND IDENTIFICATION OF RECOMBINANT PLASMID EXPRESSING HUMAN TESTIS PROSTAGLANDIN D SYNTHASE  
J.C. Lu, Y.F. Huang, X.R. Zhang

P3/4-039  
IDENTIFICATION OF PROSAPOIN DOMAINS INVOLVED IN ITS LYSOSOMAL TRAFFICKING  
D. Ham, C.R. Morales

P3/4-040  
INSL3 GENE MUTATIONS NOT RELATED TO HUMAN CRYPTORCHIDISM  
E. Moro, P. Marin, A. Ferlin, A. Garolla, C. Foresta
**Poster Session 3/4**  
Sunday, June 17

**Please note:** Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

**P3/4-041** STIMULATION OF HYPOXIA-INDUCIBLE FACTOR 1 ALPHA (HIF-1α) PROTEIN EXPRESSION IN THE ADULT RAT TESTIS FOLLOWING ISCHEMIA OCCURS WITHOUT AN INCREASE IN HIF-1α mRNA  
J. Powell, R. Elshtein, D.J. Forest, M.A. Palladino

**P3/4-042** MOLECULAR MECHANISMS GOVERNING NEUTROPHIL RECRUITMENT TO SUBTUNICAL VENULES FOLLOWING ISCHEMIA/REPERFUSION OF THE MURINE TESTIS  
J.J. Lysiak, J.L. Kirby, T.T. Turner

**P3/4-043** LOCALIZATION OF DAZ mRNA IN HUMAN TESTIS USING REVERSE TRANSCRIPTION IN SITU PCR TECHNIQUE (RT-ISHPCR)  
J.B. Warchol, A.G. Jankowska, S. Augustyniak, D. Stecewicz

**P3/4-044** DYNAMICS OF TESTICULAR HORMONE-SENSITIVE LIPASE (HSL) DURING POST NATAL DEVELOPMENT IN GUINEA PIG (GP)  
O. Kabbaj, C. Holm, M.L. Vitale, R.M. Pelletier

**P3/4-045** CAVEOLIN AND FLOTILLIN CONTENT OF RAT TESTIS PERITUBULAR MYOID CELLS AND THEIR DETERGENT-INSOLUBLE GLYCOSPHINGOLIPID ENRICHED MEMBRANE MICRODOMAINS  
M.A. Shubert, M.C. Pizzorno, S.E. Nyquist

**P3/4-046** THE LOCALIZATION OF IGF-1 BY IMMUNOHISTOCHEMICAL IN BOAR TESTES DURING PREPURITY  

**P3/4-047** MODULATION OF GLUTATHIONE LEVEL IN RAT TESTICULAR MITOCHONDRIA BY HYPOTHYROIDISM  
G.B.N. Chainy, S. Choudhury, L. Samanta

**P3/4-048** HORMONAL ALTERATIONS AND SPERMATOGENIC ARREST UNDER CAFFEINE ADMINISTRATION IN RATS  
M. Arabi, S. Oryan, K. Parivar

**Differentiation/Development**

**P3/4-049** A NOVEL MUTATION OF ANDROGEN RECEPTOR ENCODING GENE IN PATIENT WITH PARTIAL ANDROGEN INSENSITIVITVITY SYNDROME  
B. Zilaitiene, R.T. Preiksa, V. Matulevicius, H. Leffers, N.E. Skakkebaek

**P3/4-050** MODULATION OF TESTICULAR HORMONE SENSITIVE LIPASE (HSL) PROTEIN LEVELS AND ACTIVITY, AND OF TUBULAR AND INTERSTITIAL CHOLESTEROL AND TRIGLYCERIDES (TG) IN RELATION TO SERUM HORMONE PROFILES DURING PUBERTY AND THE ANNUAL SEASONAL REPRODUCTIVE CYCLE IN THE ADULT MINK (*Mustela vison*)  
O. Kabbaj, M.L. Vitale, C. Holm, J. Rose, R.M. Pelletier

**P3/4-051** ISOLATION OF PRIMORDIAL GERM CELLS FROM PIG FETUSES  
T.P. Pridantseva, I.K. Abdrakhmanov, I.P. Savtchenkova
P3/4-052  SECRETION OF LEPTIN FROM ADIPOSE TISSUE OBTAINED FROM DIFFERENT LOCATIONS BEFORE AND AFTER PUBERTY IN THE MALE RAT
S.J. Nazian

P3/4-053  CRYPTORCHIDISM AND HYPOSPADIAS RATES IN THE NETHERLANDS
F.H. Pierik, A. Burdoff, R. Juttmann, M. Vlasblom, R. Nijman, S. De Muinck Keizer, R. Weber

P3/4-054  STEROIDOGENIC CHARACTERISTICS OF HUMAN LEYDIG CELL PRECURSORS (hLCP) DURING INFANCY
E. Pellizzari, S. Meroni, M. Musse, H. Chemes, S. Cigorraga

Male Hypogonadism

P3/4-055  TESTOSTERONE UNDECANOATE (TU) HAS THE SAME EFFICACY AS TESTOSTERONE ENANTHATE (TE) WITH FEWER INJECTIONS IN THE TREATMENT OF MALE HYPOGONADISM

P3/4-056  HEMOGLOBINOPATHIES AND GONADAL AXIS IN MALES: A CROSS-SECTIONAL, MULTI-CENTER, CLINICAL STUDY IN A GREEK POPULATION
D.G. Goumis, J. Papadimas, G. Georgiadis, V. Zournatzi, J. Tzatzitsis, G. Bontis

P3/4-057  TESTOSTERONE THERAPY INDUCES SOMATIC AND DENSITOMETRIC CHANGES IN MALE HYPOGONADISM
S.M. Aszpís, G. Geloso, S. Karlsbrum, P. Otero, L. Schurman, H. Salerni, O. Levalle

P3/4-058  CIRCADIAN OSCILLATIONS OF MELATONIN (MEL) AND PITUITARY-GONADAL AXIS HORMONES IN MALES WITH CHRONIC ACTIVE HEPATITIS B (CAH) AND LIVER CIRRHOSIS (LC):
B. Marek, D. Kajdan i uk, B. Kos-Kuda, Z. Ostrowska, L. Siemiska

P3/4-059  ANDROPAUSE AND ORAL TESTOSTERONE REPLACEMENT THERAPY DESCRIPTION OF OUR EXPERIENCE IN 76 PATIENTS WITH PRIMITIVE HYPOGONADISM

P3/4-060  CHROMOSOME ANALYSIS IN BROTHER CASES OF KALLMANN’S SYNDROME, USE OF FLUORESCENCE IN SITU HYBRIDIZATION, COMPARATIVE GENOMIC HYBRIDIZATION, AND MULTICOLOR SPECTRAL KARYOTYPING
T. Kobayashi, Y. Joe, Y. Tokunaga, M. Morioka, H. Tanaka

P3/4-061  INHIBIN B AND SPERMATOGENESIS IN HYPOGONADOTROIC HYPOGONADISM TREATED WITH HCG AND RECOMBINANT FSH
A.A. Sinisi, D. Pasquali, D. Esposito, A. Notaro, A. Bellastella

P3/4-062  THE REPRODUCTIVE AND SEXUAL FUNCTIONS IN UREMIC MALES
A.M. Attia, S.H. Kandil

P3/4-063  SUCCESSFUL TREATMENT OF MALE HYPOGONADISM WITH TESTOSTERONE UNDECANOATE (TU) INJECTIONS IN EXTENDED INTERVALS OF 12 WEEKS
S. v. Eckardstein, D. Büchter, E. Nieschlag
Please note: Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

Male Sexual Dysfunction 1

P3/4-064 BIOCHEMICAL DISTURBANCES IN PATIENTS WITH IMPOTENCE
A. Lecki

P3/4-065 COMPARISONS BETWEEN COUPLES WITH DIFFERENT SMOKING HABITS: MALE AND FEMALE SEXUAL BEHAVIOR
P.M. Zavos, C.N. Zarmakoupis, J.R. Correa, K. Kaskar, P.N. Zarmakoupis-Zavos

P3/4-066 PREDISPOSING FACTORS FOR THE TRAUMATIC PENILE RUPTURE
A.F. De Rose, M. Giglio, G. Carmignani

P3/4-067 ERECTILE FUNCTION OF BICYCLING POLICE OFFICERS
S.M. Schrader, M.J. Breitenstein, J.C. Clark, B.D. Lowe, T.W. Turner

P3/4-068 THE EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON SEXUAL FUNCTION
M. Monga, M. Kamarei

P3/4-069 DEHYDROEPIANDROSTERONE SULPHATE (DHEAS) AND THE LIPID STATUS IN THE ERECTILE DYSFUNCTION
P. Kumanov, A. Tomova

P3/4-070 SEXUAL DYSFUNCTION IN COMBAT VETERANS WITH POST-TRAUMATIC STRESS DISORDER
D.J. Cosgrove, J.E. Bernie, Z. Gordon, M. Stein, M. Monga

P3/4-071 CLITORAL NITRIC OXIDE SYNTHASE ISOFORMS IN A SWINE CLITORAL CELL CULTURE MODEL
M. Rajasekaran, V. Pagnon, M. Monga

P3/4-072 PERMANENT PROLONGATION OF THE PENIS: ELSEWEIFI I TECHNIQUE
A. El-Seweifi

P3/4-073 PERMANENT THICKENING OF THE PENIS: ELSEWEIFI II TECHNIQUE
A. El-Seweifi

P3/4-074 CARBON DIOXID (CO2) LASER THERAPY OF Peyronie's Disease

P3/4-075 SUCCESSFUL MANAGEMENT OF HIGH-FLOW PRIAPISM WITH SUPERSELECTIVE EMBOLISATION
J. Rózsahegyi, Z. Kopá, L. Major, F. Szabó, A. Laki, G. Kovács, G. Papp

P3/4-076 PECULIARITIES OF TREATMENT OF PATIENTS SUFFERING FROM A MIXED COPULATIVE DYSFUNCTION WITH A LEADING INTEROCEPTIVE SYNDROME
T.E. Husainov, S.M. Kusymzhanov, A.I. Izbasarov, E.S. Ismoldaev, S.G. Zazulevsky, T.M. Tulenov

P3/4-077 PRACTICAL TREATMENT WITH VEP DEVICE
D. Bojovic, S. Bojovic

P3/4-078 SAVABO SPECIAL - IMPROVEMENT OF THE SEXUAL REFLEX
S. Bojovic, D. Bojovic, T. Trpcevski

P3/4-079 PENILE PROSTHESIS REIMPLANTATION
R. Wang, V.A. Cancellaro, J. Renehan, K. Lennox, R.W. Lewis
P3/4-080 WHAT DOES POOR RESPONSE TO INTRACavernosal Injection REALLY INDICATE?  
S.M. Elhanbly, A. Hegazy, M. Elmogy, R. Schoor, C. Niederberger, L. Ross

P3/4-081 CORRELATION OF OFFICE INJECTION TESTING (OITT) WITH HEMODYNAMIC EVALUATION: PROspectively Performed Study  
A.A. Rabea, M. Eyada, E.A. Atef, J. Mulhall, R.J. Krane, I. Goldstein

P3/4-082 CAVERNOUS NEUROTOMY CAUSES HYPOXIA AND FIBROSIS IN RAT CORPUS CavernOSUM  
S. Leungwattanakij, D.Y. Yang, J.S. Hyun, A.B. Abdel-Mageed, T.J. Bivalacqua, W.J.G. Hellstrom

P3/4-083 QUANTIFYING THE VALUE OF RESISTIVE INDEX IN THE DIAGNOSIS OF VENOUS INSUFFICIENCY  
S. Elhanbly, R. Schoor, C. Niederberger, L. Ross, M. Elmogy, A. Hegazy

P3/4-084 RESULTS OF THE USE OF COLChICINE IN THE TREATMENT OF THE TUNICA AlBuGINEA FIBROSIS  

P3/4-085 SMALL INTESTINAL SUBmUCOSA AS A TUNICA AlBuGINEA GRAFT  
M. Monga, P. Zupkas, D. Cosgrove, M. Rajasekaran

P3/4-086 RANDOMIZED, DOUBLE-BLIND, CROSSOVER STUDY OF THE COMPARATIVE PHARMACODYNAMICS OF TWO BIMix AND TWO TRIMIX INTRAcAVERNOSAL INJECTION FORMULATIONS IN PATIENTS WITH SEVERE ERRECTILE DYSFUNCTION  

P3/4-087 GENE TRANSFER OF SUpEROXIDE DISMUTASE TO THE RAT PENIS REVERSES AGE-RELATED ERRECTILE DYSFUNCTION  
T.J. Bivalacqua, H.C. Champion, P.J. Kadowitz, W.J.G. Hellstrom

P3/4-088 A COMPARATIVE STUDY TO EVALUATE THE VALIDITY OF NOCTURNAL PENILE TUMESCENCE MONITORING AND COLOR-CODED DUpLEX SONOGRAPHY IN THE DIAGNOSIS OF VASCULOGENIC IMPOTENCE  
A.H. Ahmed, M.E. Setein, S.M. Zaky

P3/4-089 SURGERY OF CORPORA CavernOSA BY Plication AND AlLODerMIC GRAFT  
F. Mantovani, E. Patelli, F. Colombo, S. Confalonieri, E. Pisan

**Immunology**

P3/4-090 SPECIFIC AGGlUTINATION OF HUMAN SPERM BY CHICKEN ANTIbODIES AGAINST HUMAN SPERM FROM EGG YOLKS OF IMMUNIZED HENS  
N.Q. Lu, Y.F. Huang, J.R. Zhao, J.P. Xu, N.G. Lu, J.W. Zhang

P3/4-091 THE ROLE OF IMMUNOSUPPRESSIVE TREATMENT IN ADOLESCENTS AND YOUNG MEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN THEIR SEMEN ANALYSIS  
F.F. Pasqualotto, J. Hallak, C.V. Locambo, S. Arap, C.A.A. Silva

P3/4-092 THE BLOOD-TESTIS BARRIER IS NOT A BARRIER TO SPERM ANTIbODIES IN THE MINK (*Mustela vison*)  
S.R. Yoon, M.L. Vitale, R.M. Pelletier
Please note: Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

P3/4-093 THE CHARACTERIZATION OF HUMAN SPERMATOZOA ANTIGENS AND IMMUNOLOGICAL INFERTILITY
C. Bohring, E. Krause, W. Krause

P3/4-094 IDENTIFICATION OF ANTIBODIES AGAINST SPERMATOZOA IN SERUM OF MALE WILD FOXES
F. Boue, B. Genin, Y. Verdier

P3/4-095 EVIDENCE FOR IMMUNOSUPPRESSIVE EFFECTS OF SEMENOGELIN, MAJOR PROTEIN OF SEMEN COAGULUM
T. Matsushita, N. Suzuki, M. Yoshiike, T. Iwamoto

P3/4-096 PROINFLAMMATORY SEMINAL CYTOKINES LEVELS IN ASYMPTOMATIC INFERTILE INFECTED MEN
E. Cardoso, I. Santioanni, A. De Paulis, S. Predari, E. Comini, A. Arregger

P3/4-097 DEVELOPMENT OF ANTIBODIES TOWARDS HUMAN ACROSIN BY GENE IMMUNIZATION
C. Veauté, L. Furlong, J. Biancotti, M. Vazquez-Levin

P3/4-098 INTERLEUKIN-1β, INTERLEUKIN-6 AND ANTI-SPERM ANTIBODIES IN SEMINAL PLASMA OF INFERTILE MEN WITH AND WITHOUT GENITAL INFECTION
A.E. Moubasher, N.A. Abdel-Wahab, A.K. Mustafa, E.F. Mohamed

P3/4-099 ORCHIDIC TOLERANCE WORKS IN THE MOUSE AS WELL
E. Veräjänkorva, N. Setälä, T. Teros, A. Liukas, A. Salmi, P. Pölänen

Assisted Reproduction

P3/4-100 SPERM MORPHOLOGY AND NUCLEAR DNA INTEGRITY AFTER DENSITY GRADIENT CENTRIFUGATION (DGC) THROUGH PURESPERM™: RELATIONSHIP TO IVF OUTCOME
M.J. Tomlinson, O. Moffatt, G.C. Manicardi, D. Bizzaro, D. Sakkas

P3/4-101 SERUM INHIBIN B CANNOT PREDICT TESTICULAR SPERM RETRIEVAL IN PATIENTS WITH NON-OBSTRUCTIVE AZOOSPERMIA

P3/4-102 SPERM RETRIEVAL AND FERTILIZATION IN REPEATED PERCUTANEOUS EPIDIDYMAL SPERM ASPIRATION

P3/4-103 CUMULATIVE PROBABILITY OF PREGNANCY AND IMPLANTATION RATES ARE SIMILAR FOR THE FIRST FOUR EMBRYO TRANSFERS (ETS) (FRESH OR FROZEN) FOLLOWING IN VITRO FERTILIZATION (IVF) WITH INTRACYTOPLASMIC SPERM INJECTION (ICSI)
J.H. Check, W. Yuen, M.L. Check, K. Swenson

P3/4-104 PERCUTANEOUS ASPIRATION BIOPSY USING AN INTRAVENOUS CATHETER: A NEW MODIFICATION FOR SPERM RETRIEVAL IN AZOOSPERMIC PATIENTS UNDERGOING ICSI
I.M. Fahmy, A. Kamal, R. Mansour, G. Serour, M. Aboulghar
P3/4-105  THE COMPARISON OF THE EFFICACY OF ICSI USING EITHER SPERMATOZOA ASPIRATED MICROSURGICALLY FROM THE EPIDIDYMIS (MESA-ICSI) OR OBTAINED FROM THE EJACULATE
S. Horak, J. Huk, K. Grettka, G. Tomanek

P3/4-106  REAL TIME SPERM MICRO-SEPARATION TECHNOLOGIES FOR HUMANS AND FARM ANIMALS
F.N. Wang, B.Y. Suh

P3/4-107  COMPARISON OF FOUR SPERM PROCESSING METHODS: EFFECT ON RECOVERY, CLEAN-UP, MOTILITY, AND MOTION PARAMETERS
C.L. Foster, W.E. Roudebusch

P3/4-108  SEMINAL TRACT WASHOUT TO TREAT INFERTILITY IN ANEJACULATING PATIENTS
G.M. Colpi, L. Negri, L. Gianaroli, M. Cristina Magli, A. Pia Ferraretti, P. Sagone

P3/4-109  EFFECT OF SEMEN PROCESSING ON CHROMATIN, MORPHOLOGY VITALITY, MOTILITY AND ITS RELEVANCE TO CRYOPRESERVATION
M.E. Hammadeh, M. Hippach, O. Neis, P. Rosenbaum, W. Schmidt

P3/4-110  COMPARISON BETWEEN SPERMATOZOA QUALITY (MORPHOLOGY AND CHROMATIN INTEGRITY) AFTER SEMEN PROCESSING WITH PURE SPERM OR GLASS WOOL FILTRATION AND THEIR EFFECT ON IVF OUTCOME
M.E. Hammadeh, M. Hippach, A. Kühnen, P. Rosenbaum, W. Schmidt

P3/4-111  ICSI IN AZOOSPERMIA WITH SURGICAL SPERM RETRIEVAL – PREDICTORS OF OUTCOME
Y. Sofer, D. Strassburger, R. Raziel, M. Schachter, O. Bern, A. Umanski, R. Ron-El, S. Friedler

P3/4-112  PREDICTIVE VALUE OF SERUM INHIBIN-β IN THE TESTICULAR ESPERMATIC RECUPERATION IN TESTICULAR AZOOSPERMIA

P3/4-113  GENETIC STUDY OF 500 STERILE COUPLES IN ICSI PROGRAMME DUE TO SEVERE MALE FACTOR
J.L. Ballescá, J. Balasch, M. Creus, L. Mengual, R. Oliva, B. Puerto, J.A. Vanrell

P3/4-114  HOW PREDICTIVE IS THE MOCK CYCLE DURING IVF; SHOULD IT BE PERFORMED?
P.M. Zavos, J.R. Correa, A.M. Sultan, K. Kaskar, P.N. Zarmakoupis-Zavos

P3/4-115  PURESPERM IS SUPERIOR TO ISOLATE DENSITY GRADIENT FOR SPERM PROCESSING
P. Ranganathan, A. Agarwal

P3/4-116  NOVEL SEMEN QUALITY SCORES CAN PREDICT PREGNANCY IN PATIENTS WITH MALE FACTOR INFERTILITY UNDERGOING INTRAUTERINE INSEMINATION

P3/4-117  NOVEL MECHANISM OF SPERM PROTECTION BY EGG-YOLK
P. Manujath, V. Nauc, M. Menard
**Prostate**

<table>
<thead>
<tr>
<th>Abstract Number</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3/4-118</td>
<td>INHIBINS, ACTIVINS AND FOLLISTATINS IN PROSTATE FUNCTION</td>
<td>G.P. Risbridger</td>
</tr>
<tr>
<td>P3/4-120</td>
<td>GAP JUNCTIONS IN HUMAN PROSTATE DISEASE</td>
<td>H. Habermann, W. Habermann, V. Ray, G.S. Prins</td>
</tr>
<tr>
<td>P3/4-121</td>
<td>CLONING OF A NOVEL ANDROGEN-REPRESSIBLE GENE EXPRESSED IN THE MOUSE PROSTATE</td>
<td>J. Singh, L. Young, D.J. Handelsman, Q. Dong</td>
</tr>
<tr>
<td>P3/4-123</td>
<td>THE GLANDULAR KALLIKREIN 2 OF THE COTTON-TOP TAMARIN IS A PSEUDOGENE</td>
<td>A.M. Olsson, K. Lundwall</td>
</tr>
<tr>
<td>P3/4-124</td>
<td>TIMM’S METHOD FOR DEMONSTRATING THE LOCALIZATION OF ZINC IN PROSTATE OF BILATERAL VASECTOMIZED RATS; LIGHT AND ELECTRON MICROSCOPIC STUDY</td>
<td>S. Cetinel, E. Ozcinar, S. Firvanc, I. Okar</td>
</tr>
<tr>
<td>P3/4-125</td>
<td>DIFFERENTIAL RESPONSE OF ANDROGEN-SENSITIVE AND ANDROGEN-REFRACTORY PROSTATE CANCER CELLS TO INDUCTION OF APOPTOSIS BY TRAIL</td>
<td>M. Eid, M.V. Kumar</td>
</tr>
<tr>
<td>P3/4-126</td>
<td>INTERMITTENT ANDROGEN SUPPRESSION (IAS) IN THE TREATMENT OF PROSTATE CANCER</td>
<td>N. Bruchovsky</td>
</tr>
<tr>
<td>P3/4-127</td>
<td>VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION FROM NEUROENDOCRINE (NE) CELLS IS ASSOCIATED WITH INVASIVE PHENOTYPE AND PATHOLOGIC STAGE RATHER THAN MICROVESSEL DENSITY (MVD) IN PROSTATE CANCER (PCA)</td>
<td>S. Andonian, V. Dam, L. Begin, S. Chevalier, A.G. Aprikian</td>
</tr>
<tr>
<td>P3/4-128</td>
<td>SOMATOSTATIN RECEPTOR mRNA IN SITU HYBRIDISATION IN HUMAN PROSTATE AND PROSTATE CANCER</td>
<td>J. Hansson, V. Gadaleanu, A. Bjartell, P.A. Abrahamsson</td>
</tr>
<tr>
<td>P3/4-129</td>
<td>BIOLOGICALLY ACTIVE PAC1 RECEPTOR ISOFORMS IN HUMAN NEOPLASTIC PROSTATE</td>
<td>C. Mammi, V. Frajese, C. Mencacci, D. Farini, G. Frajese, C. Moretti</td>
</tr>
<tr>
<td>P3/4-130</td>
<td>APOPTOSIS INDUCTION IN PROSTATIC EPITHELIAL CELLS BY ZINC IONS IN VITRO</td>
<td>H. Rumpold, G. Untergasser, G. Pfister</td>
</tr>
</tbody>
</table>
P3/4-132 THE MECHANISM OF CASODEX INDUCED CELL DEATH

P3/4-133 THE EFFECT OF THE ANTI-ANDROGEN CASODEX ON NRP-152 AND NRP-154 CELL LINES
M. Brown, C. Morrissey, M. Brush, A. Buser, N. Okezie, M. Tenniswood

P3/4-134 ANDROGENS INTERFERES WITH EGF RECEPTOR-MEDIATED SIGNAL TRANSDUCTION IN PROSTATE CANCER CELLS
L. Bonaccorsi, V. Carloni, G. Forti, E. Baldi

P3/4-135 THE QUANTITY INDICATORS MRI OF PROSTATE CANCER
S.V. Varshavski

P3/4-136 COAGULOOP TRANSURETHRAL RESECTION OF THE PROSTATE (CLITURP) AND WEDGE LOOP TURP (WLITURP) PROVIDE SAFE AND EFFICACIOUS OUTPATIENT TURP
D.J. Lehr, R.P. Kaufman, Jr.

P3/4-137 ARE PROSTATIC CONDITIONS IN CHILDREN AND TEENAGERS CRITERIA OF REPRODUCTIVE INSUFFICIENCY?

P3/4-138 PROSTATE CANCER SCREENING PROGRAM IN 2689 PATIENTS AND THE APPLICATION OF TRANSRECTAL ULTRASOUND

Clinical Urology

P3/4-139 A CONTROLLED CLINICAL TRIAL WITH THE TWO-BALLOON AND THREE-CHANNEL CATHETER IN THE THERAPY OF CHRONIC BACTERIAL PROSTATITIS
W. Huang, B. Xu, H. Wenjie

P3/4-140 MANAGEMENT OF NEGLLECTED/UNTREATED OLD CASES OF FRACTURE PENIS
A. Bhat, G. Saxena

P3/4-141 DRUG INDUCED ISOLATED PENILE SKIN GANGRENE A STUDY OF 7 CASES
A. Bhat, G. Saxena

P3/4-142 INHIBIN B EXPRESSION IN HUMAN CONGENITAL TESTICULAR PATHOLOGY

P3/4-143 β CATELIN IS OVER-EXPRESSED IN HUMAN HYPERPLASTIC LEYDIG CELLS
P. González-Peramato, D. Hardisson, J. Palacios, A. Serrano, D. Val, C.A. Suárez-Quian, J. Regadera
**Spermatogenesis 2**

P5/6-001  GERM CELL DEGENERATION IN MEIOTIC AND IN POST MEIOTIC ARREST OF SPERMATOGENESIS IN HUMAN TESTIS  
S. Francavilla, P. D'Abrizio, G. Cordeschi, G. Properzi, S. Ulisse, F. Francavilla

P5/6-002  FSH RECEPTOR ABLATION AND DELAYED SEXUAL MATURITY IN THE MALE: IMPLICATIONS FOR CONSTITUTIONAL DELAY OF PUBERTY  
H. Krishnamurthy, P.S. Babu, C. Morales, M.R. Sairam

P5/6-003  HYPOTHYROIDISM INHIBITS TESTICULAR MATURATION ON THE WAY DIFFERENT THAN ESTRADIOL DOES  
J. Slowikowska-Hilczer, K. Marchlewksa, E. Oszukowska, R. Wálczak-Jedrzejowska, K. Kula

P5/6-004  ABNORMAL SPERMATOGENESIS IN DOUBLE-MUTANT MICE WITH TARGETED DELETIONS OF TNP1 AND TNP2  
C.R. Shirley, M. Zhao, B. Mohapatra, R.R. Behringer, M.L. Meistrich, M.D. Anderson

P5/6-005  ESTABLISHMENT OF A MOUSE GERM CELL LINE EXPRESSING TIEGFR α-1 MEMBRANE RECEPTOR  
M.-C. Hofmann, E.W. Johnson, G. Dirami, L. Dettin, L.X. Feng, M. Dym

P5/6-006  CLONING AND CHARACTERIZATION OF A HAPLOID GERM CELL SPECIFIC cDNA(B390) ENCODING A NOVEL SPERM TAIL PROTEIN FROM MOUSE TESTIS  
C.E. de Carvalho, H. Tanaka, N. Iguchi, S. Ventela, Y. Nishimune

P5/6-007  CHARACTERIZATION OF HAPLOID GERM CELL SPECIFIC KINASE 'HASPIN' PROMOTER AND ITS ACTIVITY DEFINED BY TRANSGENIC MOUSE EXPERIMENTATION.  
H. Tanaka, S. Yamada, N. Yoshitake

P5/6-008  INTRACELLULAR DISTRIBUTION OF RBM7 DURING MEIOSIS AND INTERACTION WITH RNA SPlicing FACTORS  
W.A. Salameh, A. Mitchell

P5/6-009  AMH AND INHIBIN β AS MARKERS OF SERTOLI CELL FUNCTION DURING A STEROID BASED HORMONAL CONCepTIVE REGIME IN MEN  
W.M. Hair, S.C. Riley, N.P. Groome, F.C.W. Wu, G.A. Lincoln

P5/6-010  SERUM INHIBIN β VALUES REFLECT THE EFFICIENCY OF SPERMATOGENESIS IN A POPULATION OF 106 INFERTILE MEN  
J. Auger, Y. Fulla, S. Allali, L. Nonnenmacher, P. Jouannet

P5/6-011  STIMULATION OF DNA REPAIR BY THE SPERMATIDAL TP1 PROTEIN  
N. Caron, S. Veilleux, G. Boissonneault

P5/6-012  MULTIPLE KINESIN-RELATED MOTORS IN THE MAMMALIAN TESTIS  
A.O. Sperry

P5/6-013  CDK2β IS THE STARTER KINASE FOR MEIOTIC MPF DURING MOUSE SPERMATOGENESIS  

P5/6-014  IDENTIFICATION OF CKS2 AS A BINDING SUBUNIT OF THE MEIOTIC CDK2 ISOFORM, CDK2β  
E.N. Attaya, S.E. Ravnik
P5/6-015 IMPAIRMENT OF SPERMATOGENESIS AND MAST CELL PROLIFERATION IN RATS EXPOSED TO ALCOHOL DURING PREPUBERTAL PERIOD
M. Vashisht, R.K. Parshad

P5/6-016 SEMINIFEROUS EPITHELIUM CYCLE LENGTH IN DONKEYS (Equus asinus)
E.S. Neves, H. Chiarini-Garcia, L.R. França

P5/6-017 MORPHOMETRIC AND HORMONAL ASSESSMENT IN MEN WITH AZOOSPERMIA AND SERTOLI CELL-ONLY SYNDROME (SCO)
A. Rogoza, S. Wojtylak

P5/6-018 INCREASED GERM CELL PROLIFERATION IN INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) DEFICIENT MALE MICE
Y.H. Lue, A.P. Sinha Hikim, A. Leung

Spermatozoa 3

P5/6-019 IDENTIFICATION OF ANTIGENIC FOX SPERMATOZOA SURFACE PROTEINS FOR USE IN A CONTRACEPTIVE VACCINE
Y. Verdier, N. Rouet, G. Farr, F. Bou

P5/6-020 POLYSACCHARIDES CONTAINING ARABINOSE & GALACTOSE DECREASE OXIDATIVE DAMAGE OF SPERM IN VITRO
J.E. Ellington, S.A. Oliver, D.P. Evenson

P5/6-021 PROSTASOMES INHIBIT THE NADPH-INDUCED SUPEROXIDE ANION PRODUCTION AND ENHANCE THE CAPACITATION OF HUMAN SPERMATOZOA
F. Saez, G. Grizard, D. Boucher

P5/6-022 ADVERSE EFFECT OF SEMEN PROCESSING ON HUMAN SPERM DNA INTEGRITY IS NOT ASSOCIATED WITH INCREASED LEVELS OF SPERM FREE THIOLS
K.M. Kamal, K. Jarvi, D. Phang, A. Zini

P5/6-023 THE IMPACT OF OXIDATIVE STRESS ENZYMES ON SPERM MOTILITY IN PATIENTS WITH NORMAL AND ELEVATED LEVELS OF SEMINAL PLASMA LEUKOCYTES
N. Bojanic, S. Micic, N. Lalic, I. Vukovic

P5/6-024 QUALITY CONTROL OF TOTAL NON-ENZYMATIC SEMINAL ANTIOXIDANT CAPACITY BY AN ENHANCED CHEMILUMINESCENCE ASSAY

P5/6-025 ROLE OF PYRUVATE IN OXIDATIVE STRESS-INDUCED EFFECTS ON HUMAN SPERM ENERGY METABOLISM
S.C. Sikka, S.S. Shah, A. Baratta, W.J.G. Hellstrom

P5/6-026 ASSOCIATION OF POOR SPERM FUNCTION WITH LIPID PEROXIDATION AND PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS).
W.C.L. Ford, A.C. Williams
**Poster Session 5/6**

**Monday, June 18**

**Please note:** Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

P5/6-027  LEUKOCYTE-INDUCED REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN SPERM FROM LEUKOCYTOSPERMIC SAMPLES  

P5/6-028  RELATIONSHIP BETWEEN REACTIVE OXYGEN SPECIES AND 8-HYDROXYDEOXYGUANOSINE AS AN INDICATOR OF DNA DAMAGE INSPERMATOZOA  
A. Hinting, Sudjarwo

P5/6-029  ROLE OF SUPEROXIDE ANION AND HYDROGEN PEROXIDE IN ACROSOME REACTION OF BOVINE SPERMATOZOA  
C. O’Flaherty, M. Beconi, N. Beorlegui

P5/6-030  OXIDATIVE METABOLISM AND INTRACELLULAR CALCIUM VARIATION IN CAPACITATED BOVINE SPERM  
M. Córdoba, L. Pintos, M.T. Beconi

P5/6-031  FREE THIOLS (SH GROUPS) IN HUMAN SPERMATOZOA: CORRELATION WITH SPERM DNA INTEGRITY.  
K.M. Kamal, A. Zini, D. Phang

P5/6-032  BIOLOGICAL VARIABILITY OF SPERM DNA INTEGRITY IN SEMEN SAMPLES FROM INFERTILE MEN  
K.M. Kamal, D. Phang, K. Jarvi, J. Willis, A. Zini

P5/6-033  CHROMATIN STABILITY IN SEX-SORTED SPERM  

P5/6-034  REDUCED SEnescence AND RETAINED CHROMATIN INTEGRITY IN HUMAN SPERM PREPARED BY DENSITY GRADIENT CENTRIFUGATION  
J.M. Morrell, D. Sakkas, O. Moffatt, G.C. Manicardi, D. Bizzaro, P.V. Holmes

P5/6-035  RIBONUCLEIC ACID CONTENT IN SPERMATOZOA: MOTILE VERSUS NONMOTILE  
W.E. Roudebush, E.T. Purnell

P5/6-036  IMPORTANCE OF SEMEN BANKING IN PATIENTS WITH SYSTEMIC DISEASES  
P. Ranganathan, A.M. Mahran, J. Hallak, A. Agarwal

P5/6-037  ENHANCED SPERM-MEDIATED GENE TRANSFER BY ELECTROPORATION: EFFICIENCY, RELIABILITY AND EXPRESSION OF TRANSGENE  

P5/6-038  ZYMOGRAPHIC EVALUATION OF PROACROSIN/ACROSIN SYSTEM INSPERMATOZOA OF TWO MARSUPIAL SPECIES, THE BRUSHTAILPOSSUM (TRICHOSURUS VULPECULA) AND THE TAMMAR WALLABY (MACROPUS EUGENII)  
K.S. Sidhu, K.E. Mate, F.C. Moline, J.C. Rodger

P5/6-039  DIRECT EFFECTS OF MAST CELL PRODUCTS ON HUMAN SPERM MOTILITY  
S.E. Weidinger, M. Sbornik, V. Meineke, A. Mayerhofer, J. Ring, F.M. Köhn
Testis 2

P5/6-040 LOCALISATION OF PROLACTIN RECEPTORS IN THE REPRODUCTIVE TRACT OF THE RAM
K. Whittington, M. Bowden, H.D. Nicholson, D. Tortone

P5/6-041 INFLUENCE OF TESTICULAR SEROTONERGIC, MELATONINERGIC, AND CATECHOLAMINERGIC SYSTEMS ON TESTOSTERONE AND cAMP PRODUCTION
M.B. Frungieri, K. Zitta, O. Pignataro, S. Gonzalez-Calvar, R. Calandra

P5/6-042 HORMONAL REGULATION OF MONOMERIC AND DIMERIC INHIBIN PRODUCTION IN HUMAN SEMINIFEROUS TUBULES
R. Trigo, E. Pellizzari, N. Groome, S. Gottlieb, S. Cigorraga, S. Campo

P5/6-043 CRYPTORCHIDISM: SEASONAL VARIATIONS IN GREECE
C. Mamoulakis, S. Antypas, A. Stamatiadou, D. Dimitriadis, A. Tzonou, N. Sofikitis

P5/6-044 SERUM INHIBIN Β CONCENTRATION AS A DIAGNOSTIC AND PROGNOSTIC MARKER IN IDIOPATHIC OLIGOZOOSPERMIA.
D.A. Adamopoulou, N. Kapolla, S. Nikopoulou, A. Pappa, A. Gregoriou

P5/6-045 MORPHOMETRIC ANALYSIS OF THE TESTIS IN ADULT MULES
L.R. de França, H. Chiarini-Garcia, E.S. Neves

P5/6-046 PURIFICATION OF RAT LEYDIG CELLS: INCREASED YIELDS AFTER UNIT GRAVITY SEDIMENTATION OF COLLAGENASE DISPERSED INTERSTITIAL CELLS
A. Salva, G.R. Klinefelter, M.P. Hardy

P5/6-047 TESTOSTERONE PRESERVES MITOCONDRIAL RESPIRATION OF IN VITRO CULTURED RAT LEYDIG CELLS EXPOSED TO LIPOPOLYSACCHARIDE
S. Valenti, B. Guazzini, L. Fazzuoli, M. Giusti

P5/6-048 EXTRACELLULAR MATRIX PROTEINS MODULATE TESTOSTERONE PRODUCTION BY LEYDIG CELLS, IN VITRO
E.S. Diaz, E. Pellizzari, S. Meroni, S. Cigorraga, L. Lustig, B. Denduchis

P5/6-049 APOPTOSIS OF GERM CELLS IN AN EXPERIMENTAL MODEL OF AUTOIMMUNE ORCHITIS: INVOLVEMENT OF THE FAS-FAS L SYSTEM
S. Theas, C. Rival, M. Ozu, L. Lustig

P5/6-050 POSTNATAL CHANGES IN LACTATE DEHYDROGENASE OF BUFFALO TESTIS
G.S. Bilaspuri, B.K. Rana

P5/6-051 CHANGES IN PROTEINS AND ENZYMES DURING DEVELOPMENT AND MATURATION OF BUFFALO TESTIS
G.S. Bilaspuri, B.K. Rana

P5/6-052 THE EARLY CHANGES OF SEMINIFEROUS TUBULAR BOUNDARY ZONE & ELASTIC FIBERS IN VASECTOMIZED YOUNG RATS; LIGHT & ELECTRON MICROSCOPIC STUDY
E. Ozcinar, U. Uslu, S. Cetinel, I. Okar
Please note: Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

P5/6-053 KING'S 'CRISS-CROSS' SIGN IDENTIFIES MALIGNANT TUMOURS OF THE TESTIS  
S.L. Sriprasad, L. Bushby, G. Muir, W. Choi, P.S. Sidhu

P5/6-054 C-KIT DEFICIENCY NOT ONLY AFFECTS SPERM PRODUCTION BUT ALSO ALTERS SPERM FERTILISING ABILITY  
D. Royere, F. Guerif, V. Laurent Cadoret, J.J. Panthier, M.T. Hochereau de Reviers

Testicular Cancer

P5/6-055 FERTILITY AFTER CHEMOTHERAPY (CT) AND RADIOTHERAPY (RT) FOR TESTICULAR GERM CELL TUMORS  
V. Mamaladze, M. Saghirashvili, N. Gvinepadze, M. Janigava

P5/6-056 SEASONALITY OF BIRTH IN PATIENTS WITH NEOPLASIAS OF THE TESTIS AND THE PROSTATE  
A.G. Amador, G. Eckert, K. Larby

P5/6-057 FERTILITY AFTER TESTIS CANCER  

P5/6-058 HIGHER SPERM CONCENTRATION IN SOUTHERN SWEDEN AS COMPARED TO DENMARK CORRESPONDS TO A LOWER THE RISK OF TESTICULAR CANCER  
J. Richthoff, J. Malm, A. Giwercman

P5/6-059 CELLULAR MANIFESTATIONS OF UNDESCENDED TESTES DEPEND UPON THE ETIOLOGY OF CRYPTORCHIDISM BUT NOT THE ABDOMINAL LOCATION PER SE  
D.N.R. Veeramachaneni, J.S. Palmer, J.D. Palmer, C.A. Awoniyi

P5/6-060 SERTOLI CELL TUMOR ASSOCIATED WITH KLINFEILER'S SYNDROME  
S. Gottlieb, R. Rey, M. Venara, G. Bastida, G. Finkielstain, H.E. Chemes

P5/6-061 ANEUPLIOIDY IN SPERMATOZOA AFTER PEB CHEMOTHERAPY USED IN TESTICULAR CANCERS  
P. De Mas, M. Daudin , M.C. Vincent, P. Calvas, G. Bourrouillou, L. Bujan

P5/6-062 DYSGENETIC GONADS MAINTAIN PREINVASIVE GERM CELL CARCINOMA PREDOMINANTLY AT 46, XY KARYOTYPE  
J. Slowikowska-Hilczer, M. Szarras-Czapnik, T.E. Romer, K. Kula

Hormone Control

P5/6-063 RELATIONSHIP OF INSULIN, SEX HORMONES, LEPTIN AND C21 STEROIDS IN MEN WITH REPRODUCTIVE DISORDERS  
N. Goncharov, M.V. Koryakin, G.V. Katsiya, G.S. Kolesnikova, A.D. Dobracheva, T.N. Todua

P5/6-064 EXPRESSON OF LUTEINIZING HORMONE (LH) SUBUNIT GENES IN THE RAT EPIDIDYMIS AND SEMINAL VESICLE  
J-S. Paick, S.W. Kim, S.H. Lee
P5/6-065 SODIUM VALPROATE MONOTHERAPY AND SEX HORMONES IN MEN
S.K. Jain, N. Jain

P5/6-066 POSSIBLE PARTICIPATION OF PHOSPHOLIPASE A2 (PLA2) ACTIVATION ON FSH-REGULATION OF LACTATE PRODUCTION IN RAT SERTOLI CELLS
S.B. Meroni, G. Gòmez, F. Riera, E. Pellizzari, H. Schteingart, S. Cigorraga

P5/6-067 DISTURBANCES OF DAILY PITUITARY-GONADAL AND -ADRENAL AXIS HORMONES SECRETION IN MALES WITH LIVER CIRRHOSIS (LC) AND CHRONIC ACTIVE HEPATITIS B (CAH)
D. Kajdaniuk, B. Marek, Z. Ostrowska, B. Kos-Kuda, K. Wirska-Korczala

P5/6-068 HORMONAL REGULATION OF FSH POLYMORPHISM IN ANORCHID BOYS
V. Ambao, S. Creus, S. Gottlieb, S. Campo

P5/6-069 SIGNIFICANT REGIONAL DIFFERENCES IN SEXUAL HORMONE CONCENTRATION IN BLOOD SAMPLES OF MILITARY CONSCRIPTS
B. Zilaitiene, N. Jorgensen, A.G. Andersen, N.E. Skakkebaek, V. Matulevicius, R. Zalinkevicius

P5/6-070 THE POSSIBLE ROLE OF PROLAKTIN IN REGULATION OF ADRENAL ANDROGEN FUNCTION
M. Koryakin, A.S. Akopyan, N.P. Goncharov

P5/6-071 EFFECTS OF LEUPROLIDE TREATMENT ON PLASMA FSH LEVELS AND TESTIS DEVELOPMENT
D.C. de Melo Rocha, J.R. Miranda, L. Debeljuk, L.D. Russell, L.R. França

Ageing

P5/6-072 INCREASED PROCOLLAGEN α1 (III) EXPRESSION IS AN EARLY INDICATOR OF FIBROSIS IN THE AGING PENIS
M. Rajasekaran, A. Kasyan, M. Monga

P5/6-073 HORMONE REPLACEMENT THERAPY FOR PADAM: OUTCOME AND SAFETY (MIDDLE EAST MULTICENTER STUDY)
H. Ghanem, A. El-Meliegy, M. Mansi, N. Younis, K. Al-Shoumer, S. Al-Rubaei, S. Merhej

P5/6-074 MAST CELL IN THE AGING HUMAN TESTIS
H. Rodriguez , A. Irsula, G. Diaz, E. Ossandon

P5/6-075 INCREASE IN MISCARRIAGE RISK WITH MATERNAL AND PATERNAL AGE: RESULTS OF A MULTICENTER EUROPEAN STUDY
P.F. Thonneau

P5/6-076 EFFECT OF AGEING AND HORMONAL REPLACEMENT THERAPY ON RAT TESTES: LINKS WITH APOPTOSIS, MITOCHONDRIAL FUNCTION AND OXIDATIVE STRESS
S.O. Nassar, N.E. Elashmawy, S.A. Elsawi , M.M. Attia

P5/6-077 AN EQUATION FOR THE CALCULATION OF BIOAVAILABLE TESTOSTERONE
M.T. Haren, B.E.C. Nordin, J.E. Morley, G.A. Wittert
**Poster Session 5/6**

**Monday, June 18**

**Please note:** Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

**P5/6-078** SERUM TESTOSTERONE LEVELS IN PROVEN FERTILE, NORMOZOOSPERMIC MEN: A BETTER GROUP FOR NORMAL VALUES?
M.S. Bornman, M. Roux, W. Vermaak, S. Reif

**P5/6-079** SERUM LEVELS OF ADRENAL AND TESTICULAR STEROIDAL HORMONES IN AGED MALES
S. Kitahara, M. Miyazaki, M. Yano, C. Nakajima, K. Yasuda, K. Yoshida

**P5/6-080** AGE-RELATED ALTERATIONS IN MALE ERECTILE FUNCTION: BROWN-NORWAY RAT MODEL
M. Rajasekaran, D. Cosgrove, A. Kasyan, M. Monga

**P5/6-081** REJUVENATION OF AGED LEYDIG CELLS IS POSSIBLE IN A RODENT SPECIES
S.M.L.C. Mendis-Handagama, I-S.Kim, H.B.S. Ariyaratne

**P5/6-082** POWER TRAINING AND TESTOSTERONE SUPPLEMENTATION IN OLDER ANDROGEN DEFICIENT MEN

**P5/6-083** THE MOLECULAR BIOLOGY AND PATHOLOGY OF THE AGING RAT VENTRAL PROSTATE
J. Scolaro, C. Morrissey, A. Buser, J. O'Sullivan, A. Moquin, M. Tenniswood

**P5/6-084** SUPRAPHYSIOLOGIC ANDROGEN ADMINISTRATION IN ELDERLY MEN UNDERGOING JOINT REPLACEMENT SURGERY
J.K. Amory, H.C. Chansky, B.D. Anawalt, A.M. Matsumoto, W.J. Bremner

**P5/6-085** ANDROPAUSA COUNSELLING IN EASTERN HUNGARY
G. Bógyi, E. Viktor, Z. Kassai, B. Csapó, A. Borsos

**Toxicology**

**P5/6-086** THE INCIDENCE FOR INTAKE OF ANIMAL PRODUCTS WITH PHARMACOLOGICAL ACTIVE RESIDUES OF ZERANOL AND ITS POSSIBLE EFFECT ON MALE FERTILITY
S. Grobler, J. Pieterse, P. van der Merwe, P.H. Wessels, G. Joubert

**P5/6-087** THE EPIDIDYMIS: A TOXICOLOGICAL ANALOG OF THE KIDNEY?
S.B. DuTeaux, M.G. Miller

**P5/6-088** THE EFFECT OF CHRONIC SMOKING ON SEMINAL PLASMA INSULIN-LIKE GROWTH FACTOR-1 IN IDIOPATHIC OLIGO-ASTHENO-TERATOZOOSPERMIA (OAT SYNDROME)
A.H. Ahmed, S.M. Abo-Azma, S. Fayed

**P5/6-089** CONTRIBUTION OF ENVIRONMENTAL FACTORS TO THE RISK OF MALE INFERTILITY
A. Oliva, A. Spira, L. Maltigner

**P5/6-090** THE COURSE EFFECTS OF DIETHYLSILBESTROL (DES) ON FERTILITY OF ADULT MALE RATS
H.O. Goyal, T.D. Braden, A. Atkinson, M. Mansour, C.S. Williams, A. Robateau

**P5/6-091** SERTOLI CELL INJURY IN ETHANOL-TREATED RATS: NOVEL ROLES FOR APOPTOSIS-RELATED GENES
N.A.S. Eid, Y. Ito, M. Shibata, K. Kusakabe, H. Abe, Z.L. Li, Y. Otsuki

72 | VIIth International Congress of Andrology
P5/6-092 SCREENING AND TESTING METHOD FOR ENDOCRINE DISRUPTORS - RODENT 20-DAY THYROID/PUBERTAL ASSAY

P5/6-093 RNA SYNTHESIS IN HUMAN TESTIS: INTERFERENCE BY PARATHION
H. Rodriguez, K. Walker, M. Guzman, E. Bustos-Obregon

P5/6-094 REPRODUCTIVE TOXICOLOGY OF ETHANOL IN MALE RATS
W.D. Kempinas, S.U. Oliva, A.G. Messias

P5/6-095 POSSIBLE ROLE OF REACTIVE OXYGEN SPECIES ON HUMAN SPERM QUALITY AND FUNCTION
M. Huang, F. Xing

P5/6-096 POLYCHLORINATED BIPHENYL (AROCJLOR 1254) TREATMENT CAUSES ASPERMIA IN RHESUS MONKEY (MACACA MULATTA)
U. Ahmad, S. Tariq, S. Jalali, M. Shahab, M.M. Ahmad

P5/6-097 PESTICIDE EXPOSURE AND FERTILITY: RESULTS OF AN EPIDEMIOLOGICAL STUDY OF FRENCH VINEYARD WORKERS
P.F. Thonneau, B. Ducot, L. Multigner, A. Schweitzer, M. Wagner, A. Clavert

P5/6-098 LEAD AND CADMIUM CONCENTRATIONS IN SEMINAL PLASMA AND COMPARISON WITH SEMINAL QUALITY IN HEALTHY KOREAN ARMY RECRUITS
K.H. Rha, W.H. Lee, Y.D. Choi, S.W. Han, M.S. Lee

P5/6-099 LEAD (Pb²⁺) INDUCES EPIGENETIC MODIFICATION OF RAT TESTICULAR GENE EXPRESSION: A DNA MICROARRAY STUDY
C. Millan, R. Sokol, Q. Shi, I.R. Hurley, G.M. Centola, S. Benoff

P5/6-100 FAS SIGNALING SYSTEM MEDIATES CADMIUM-INDUCED TESTICULAR APOPTOSIS IN RATS THE POSSIBLE PROTECTIVE ROLE OF SELENIUM
W.M. Awara, N.E. El-Ashmawy, S.O. Nassar, S.M. Talaat

P5/6-101 ENVIRONMENTAL POLLUTION AFFECTS NUMBER, VIABILITY AND MOTILITY OF SPERMATOZOA: A STUDY BY C.A.S.A.
M. De Rosa, U. Carbone, L. Paesano, S. Zarrilli, B. Amalfi, F. Cimmino, A. Colao, G. Lombardi

P5/6-102 EFFECTS OF CASTRATION ON LINDANE MEDIATED HEPATOTOXICITY
D.D. Bansal, R. Ghosh, R. Sharma, S. Puri

P5/6-103 EFFECTS OF IN UTERO EXPOSURE TO TRIBUTYL Tin ON SPERM MOTILITY IN ADULT RATS
M. Gregory, J. Barthelemy, A. Adeeko, D. Li, D.G. Cyr

P5/6-104 EFFECTS OF DIOXIN ON PENILE ERECTION IN THE RABBIT
J-J. Kim, H.S. Park, Y.S. Kim, D.G. Moon

P5/6-105 EFFECT OF IN UTERO EXPOSURE TO TRIBUTYL Tin ON FETAL RAT TESTIS
O. Kishta, L. Hermo, D. Li, A. Adeeko, C.R. Morales
**Male Sexual Dysfunction 2**

**P5/6-108**
**EFFICACY OF SILDENAFIL VS INJECTION THERAPY FOR PSYCHOGENIC ERECTILE DYSFUNCTION (ED) CASES**
A.A. Rabea, M.M. Eyada, A.I. Elakhras

**P5/6-109**
**ERECTILE DYSFUNCTION- ROLE OF SILDENAFIL SALVAGE THERAPY**
V. Bhatia

**P5/6-110**
**SILDENAFIL MONOTHERAPY VERSUS POLYPHARMACOTHERAPY FOR ERECTILE DYSFUNCTION**
V. Bhatia

**P5/6-111**
**ERECTILE DYSFUNCTION POST RADICAL PELVIC SURGERY: MANAGEMENT WITH SILDENAFIL AND L-ARGININE EVALUATED BY BUCKLING - TEST**
F. Mantovani, E. Patelli, F. Colombo, F. Pozzoni, E. Pisani

**P5/6-112**
**ORAL SILDENAFIL IN THE TREATMENT OF ARTERIOGENIC IMPOTENT PATIENTS**
M. Mancini, L. Negri, G. Piediferro, G.M. Colpi

**P5/6-113**
**PREVALENCE OF ERECTILE DYSFUNCTION AND SILDENAFIL USE IN CALIFORNIA**
M. Monga, R. Bettencourt, E. Barrett-Connor

**P5/6-114**
**EFFECTS OF SILDENAFIL ON SEMEN QUALITY AND MALE ACCESSORY GENTAL GLAND (MAGG) FUNCTION**
N. Kanakas, M. Melekos, N. Sofikitis

**P5/6-115**
**EFFECTS OF SILDENAFIL ON SLEEP-RELATED ERECTIONS IN NORMAL MEN**
G. Antonio, B. Madeo, A. Balestrieri, V. Rochira, C. Carani

**P5/6-116**
**ALFUZOSIN PHARMACOTHERAPY IN PRIMARY PREMATURE EJACULATION**
V. Bhatia

**P5/6-117**
**THERAPEUTIC ROLE OF SHORT INTRACAVERNOUS ALPROSTADIL IN ARTERIOGENIC IMPOTENT PATIENTS**
L. Negri, M. Mancini, G.M. Colpi, M. Maggi, G. Forti

**P5/6-118**
**COMPARATIVE STUDY OF EFFECT OF DOPTHIEPIN, TRAZADONE AND FLUOXETINE IN TREATMENT OF IMPOTENCE SECONDARY TO DEPRESSION**
B. Sharma, C.M. Sharma
P5/6-119 COMPARISON OF VASOACTIVE NEUROPEPTIDES IN THE HUMAN PENIS AND CLITORIS  
A. Jungwirth, C. Hauser-Kronberger, K. Fink, N. Schmeller

P5/6-120 YOHIMBINE TREATMENT OF ORGANIC ERECTILE DYSFUNCTION (ED) IN A DOSE ESCALATION TRIAL  

P5/6-121 THE EFFECT OF NATURAL OIL AS A SOURCE OF GAMMA LINOLENIC ACID ON ERECTILE DYSFUNCTION IN PATIENTS OF DIABETES MELLITUS  
C.M. Sharma, B. Sharma

P5/6-122 INTRACavernous injection test with prostaglandin E-1. our experience  

P5/6-123 INTRACavernosal vasoactive agents induce cytotoxicity in cultured human penile cavernosal smooth muscle  
M. Monga, V. Pagnon, M. Rajasekaran

P5/6-124 CHOLINERGIC STIMULATION OF PENILE ERECTION IN MICE  

P5/6-125 MECHANISM OF RELAXATION OF RABBIT CAVERNOSA SMOOTH MUSCLES BY ESTROGEN  
S.C. Kim, K.K. Seo, Y.K. Seo, M.Y. Lee

P5/6-126 CIALIS™ (IC351): EFFECTIVE AND WELL-TOLERATED TREATMENT FOR ED  
G. Brock, J. Iglesias, K. Toulouse, K. Ferguson, W. Pullman, G. Anglin

P5/6-127 EFFECTS OF DIOXIN ON PENILE ERECTION IN THE RABBIT  
DG. Moon, Y.W. Kim, H.S. Park, J.B. Choi, Y.S. Kim, J.J. Kim

P5/6-128 BISPHERALA INHIBITS PENILE ERECTION VIA ALTERATION OF PENILE HISTOLOGY IN THE RABBIT  
DG. Moon, H.S. Park, J.B. Choi, Y.S. Kim, J.J. Kim

P5/6-129 TRANSPENILE DELIVERY OF MICROVASCULOKINETIC DRUGS (TRAPS) IN THE MEDICAL TREATMENT OF ERECTILE DYSFUNCTIONS FOR PENILE REHABILITATION  
J. Tritto

Male Fertility and Infertility 2

P5/6-130 IS ABNORMAL FSH PRECLUDING PATIENTS FROM DOING TESTIS BIOPSY OR TESE?  
M. Moein, M.A. Khalili

P5/6-131 TREATMENT OF IDIOPATHIC OLIGOZOOSPERMIA WITH rh-FSH  
C. Foresta, M. Merico, M. Rossato, D. Spolaore, A. Bettella

P5/6-132 DOES CLOMIPHENE CITRATE IMPROVE FERTILIZING POTENTIAL OF SPERMATOZOA?  
J.S. Gokral, P.K. Meherji, K. Gopalkrishnan, R. Shah, V. Kulkarni

P5/6-133 INHIBIN B IS A HORMONAL MARKER OF THE ADVERSE EFFECT OF CRYPTORCHIDISM ON SPERMATOGENESIS  
Please note: Even numbered abstracts will be presented in the morning session (11:00 - 12:30), and odd numbered abstracts will be presented in the afternoon (13:30 - 15:00).

P5/6-134 CORRELATION BETWEEN SEX HORMONES AND PARAMETERS OF SPERMOGRAM IN MALE ACCESSORY GLAND INFECTION
R. Burnazyan

P5/6-135 THE FUNCTION OF MEN REPRODUCTIVE SYSTEM, WHICH WERE PREPARED FOR PROGRAM EXTRA - CORPORAL FERTILIZATION

P5/6-136 ANALYSES OF SPERM QUALITY IN LARGE POPULATIONS OVER DECADES BY DATAMINING AND DATAWAREHOUSING (WINSPEMÆ)
U. Paasch, C. Thieme, H. Glander

P5/6-137 MALE INFERTILITY RISK FACTORS IN A FRENCH MILITARY POPULATION (RESULTS OF A CASE-CONTROL STUDY)
P.F. Thonneau, J.F. Velez de la Calle, M.T. Le Martelot, B. Ducot

P5/6-138 DOES MALE SEXUAL DYSFUNCTION INFLUENCE MALE INFERTILITY?
F.X.A. Adimoejia, H. Basuki

P5/6-139 TEAM MANAGEMENT OF MALE INFERTILITY FROM SPINAL CORD INJURY
S.T. Hill, T.C. Lim, D.J. Brown, N. Cooper, H.W.G. Baker

P5/6-140 TRANSRECTAL ULTRASOUND GUIDED SEMINAL VESICULOGRAPHY IN EVALUATION OF AZOSPERMIC PATIENT
T.A. Azm, E.H. Sherif, S.O. Samir

P5/6-141 MEASURING MALE FERTILITY
J. Olsen

P5/6-142 TIME TO PREGNANCY AND SEMEN PARAMETERS: A CROSS SECTIONAL STUDY AMONG FERTILE COUPLES FROM FOUR EUROPEAN CITIES

P5/6-143 VASOEPIDIDYMOSTOMY AND VASOVASOSTOMY ARE ASSOCIATED WITH RETENTION OF SPERM CYTOPLASMIC DROPLETS
B.H. Chew, K. Jarvi, K. Kamal, J. Willis, A. Zini

P5/6-144 4-D ENDORECTAL ULTRASOUND IN DIAGNOSIS OF OBTRURATIVE DECREASE OF FERTILITY
A.P. Wieczorek, E. Korobowicz, G. Jakiel, S. Bakalczuk, M. Przytula -Pilat, M. Bokiniec, M. Semczuk

P5/6-145 IS THERE A DIFFERENCE BETWEEN DIFFERENT INTERVALS AFTER VASECTOMY AND THE REPRODUCTIVE CAPACITY FROM VASECTOMYZED MEN?

P5/6-146 EVALUATION OF THE EFFICACY OF TRANSRECTAL ULTRASOUND IN THE DIAGNOSIS OF EJACULATORY DUCT OBSTRUCTION IN INFERTILE MEN
M.T. Anis, M. El-Gammal, A. Meshref, O. Abdel-Razzak, A. Selim
<table>
<thead>
<tr>
<th></th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5/6-147</td>
<td>ANTISPERM ANTIBODIES RECOGNISE PROSTASOMES</td>
<td>L. Carlsson, C. Allegrucci, G. Ronquist, O. Nilsson, A. Minelli</td>
</tr>
<tr>
<td>P5/6-148</td>
<td>SIMPLE METHOD TO PREDICT IVF OUTCOME</td>
<td>Y. Erenpreiss, J. Erenpreisa, Y. Zalkalns</td>
</tr>
<tr>
<td>P5/6-149</td>
<td>PHOSPHATIDYLINOSITOL 3-KINASE INHIBITION ENHANCES HUMAN SPERM MOTILITY IN OLIGOASTHENOSPERMIC PATIENTS</td>
<td>M. Luconi, L. Gandini, A. Lenzi, E. Filimberti, G. Forti, E. Baldi</td>
</tr>
<tr>
<td>P5/6-150</td>
<td>MICROBIOLOGICAL STUDY OF SEMEN FROM ASYMPTOMATIC INFERTILE MEN</td>
<td>S.J. Penna Vide, A. Padrón-Medina, M. Reggis, J. Guillén, C. González, J.R. Cermeño-Vivas</td>
</tr>
<tr>
<td>P5/6-151</td>
<td>PREVALENCE AND TREATMENT OUTCOME OF BACTERIAL INFECTION IN THE SEMEN OF INFERTILE MEN</td>
<td>K.M. Kamal, K. Jarvi , A. Zini</td>
</tr>
<tr>
<td>P5/6-152</td>
<td>GENITAL MYCOPLASMAS AND ITS IMPACT ON SPERM SAMPLES IN INFERTILE MEN</td>
<td>J. Rojas-Retis, C. Bravo-Gatica, R. Salas, J. Moreno-Aranda, R. Tapia-Serrano</td>
</tr>
<tr>
<td>P5/6-153</td>
<td>GENOMIC ORGANIZATION OF THE SP22 GENE AND A UNIQUE PATTERN OF EXPRESSION IN SPERMATOGENIC CELLS</td>
<td>J.E. Welch, R.R. Barbee, J.D. Suarez, N.L. Roberts, G.R. Klinefelter</td>
</tr>
</tbody>
</table>
PP-001 ANDROGEN SELECTIVITY OF NATURAL DIRECT REPEAT RESPONSE ELEMENTS
B. Haendler, K. Barbulescu, I. Schuttke, C. Geserick, W.D. Schleunling

PP-002 ENDOTHELIAL CELL PROLIFERATION AND EXTRACELLULAR MATRIX PRODUCTION ARE INCREASED BY HYPERPLASTIC HUMAN LEYDIG CELLS

PP-003 INJECTABLE TESTOSTERONE UNDECANOATE (TU) WITH ORAL OR INJECTABLE NORETHISTERONE (NET) PREPARATIONS FOR MALE CONTRACEPTION

PP-004 TESTOSTERONE UNDECANOATE (TU) MAINTAINS SPERM SUPPRESSION INDUCED BY CYPROTERONE ACETATE (CPA) PLUS TU
A. Costantino, S. Cerpolini, W.J. Bremner, C. Flamigni, M.C. Meriggiola

PP-005 DELIVERY OF ANTIBODIES FOR MALE IMMUNOCONTRACEPTION

PP-006 CONGENITAL BILATERAL ABSENCE OF THE VAS DEFERENS (CBVD) : CLINICAL AND SEMINAL PARAMETERS, AND CFTR GENE MUTATIONS
R. Mieusset, M. Daudin, E. Bieth, L. Bujan, F. Pontonnier

PP-007 DAX-1 REPORTER TRANSGENE EXPRESSION IN MOUSE TESTES
R. Behdjani, N. Pilon, I. Daneau, D.W. Silversides

PP-008 THE NATRIURETIC PEPTIDES STIMULATE STEROIDOGENESIS IN THE FETAL RAT TESTIS
F. El-Gehani, M. Tena-Sempere, H. Ruskoaho, I. Huhtaniemi

PP-009 A NOVEL MUTATION IN THE D-BOX OF THE ANDROGEN RECEPTOR GENE (S597R) IS ASSOCIATED WITH BOTH PAIS AND NORMAL PHENOTYPE
Y.L. Giwercman, J. Richthoff, S. Ivarsson, J. Malm, A. Giwercman

PP-010 OXYTOCIN INCREASES ACTIVITY OF BOTH ISOFORMS OF 5ALPHA-REDUCTASE IN THE MOUSE EPIDIDYMIS
H.D. Nicholson, K. Mirfin

PP-011 WITHDRAWN

PP-012 IMMORTALIZED EPIDIDYMAL CELL LINES FROM TRANSGENIC MICE HARBORING TEMPERATURE-SENSITIVE SV 40 LARGE T ANTIGEN GENE
Y. Araki, K. Suzuki, R.J. Matusik, M. Obinata, M-C. Orgebin-Crist

PP-013 EPIDIDYMIS-SPECIFIC SPERM BINDING PROTEINS

PP-014 HOX TRANSCRIPTION FACTORS AND SEGMENTAL FUNCTION OF THE ADULT EPIDIDYMIS
D. Bomgardner, B.T. Hinton, T.T. Turner
Tuesday, June 19

President's Poster Session

PP-015 DIFFERENTIAL REGULATION OF GENE EXPRESSION ALONG THE EPIDIDYMIS AFTER ORCHIDECTOMY
N.N. Ezer, B. Robaire

PP-016 EVENING MELATONIN FURTHER INCREASES ADULT TESTES SIZE AND GERM CELL NUMBER IN NEONATAL HYPOTHYROID RATS
A.V. Ramachandran, S.K. Lagu, N.G. Bhavsar

PP-017 HIGH INCIDENCE OF SINGLE NUCLEOTIDE SUBSTITUTIONS IN THE MITOCHONDRIAL GENOME IS ASSOCIATED WITH POOR SEMEN PARAMETERS IN MAN

PP-018 PREDICTING PREGNANCY AND SPERMATOGENESIS BY SURVIVAL ANALYSIS DURING GONADOTROPIN TREATMENT OF GONADOTROPIN DEFICIENT INFERTILE MEN
P.Y. Liu, L. Turner, A.J. Conway, S. Wishart, D.J. Handelsman

PP-019 LOCALIZATION, FERTILITY INHIBITION, AND EPITOPE MAP USING ANTIBODIES TO THE SPERM PROTEIN SP22

PP-020 ENVIRONMENTAL FACTORS AND ERECTILE DYSFUNCTION
A. Oliva, A. Giami, L. Multigner

PP-021 SILDENAFIL DOES NOT IMPROVE ERECTIONS IN YOUNG MEN WITH NORMAL SEXUAL FUNCTION: RESULTS OF A RANDOMIZED DOUBLE-BLIND, PLACEBO CONTROLLED CLINICAL STUDY
N. Mondaini, R. Ponchietti, F. Di Loro, S. Biscioni, A. Bongini, M. Rizzo

PP-022 DIFFERENTIATION OF LEYDIG CELLS AND PERITUBULAR CELLS IN FETAL HUMAN TESTIS FROM 17TH TO 23RD POSTCONCEPTIONAL WEEK
S.G. Haider, G. Berthold, R. Darbandi

PP-023 EX VIVO EXPRESSION OF VASCULAR ENDO THELIAL GROWTH FACTOR AND ITS RECEPTORS IN HUMAN PENILE CAVERNOSAL CELLS
M. Rajasekaran, A. Kasyan, M. Monga

PP-024 REGULATION OF rho-kinase MEDIATED VASOCONSTRICTION IN THE PENILE CIRCULATION
T. Mills, H. Branam, K. Chitaley, P. Jackson, R. Lewis, V. Stopper, C. Webb, C. Wingard

PP-025 EPIGENETIC CHANGES OF ANDROGEN RECEPTOR GENE IN HUMAN PROSTATE CANCER

PP-026 SELECTIVE MOLECULAR ABLATION OF EPITHELIAL TISSUES IN CANINE PROSTATE: DEVELOPMENT OF PROSTATE-SPECIFIC ANTIGEN PROMOTER-BASED SUICIDE GENE THERAPY FOR BENIGN PROSTATIC HYPERPLASIA

PP-027 PROSTATE AND TESTIS ARE RICH SOURCES FOR GLYCOPROTEIN HORMONE SUBUNITS IN HUMAN SEMEN
PP-028  LIGAND-INDEPENDENT ACTIVATION OF THE ANDORGEN RECEPTOR AND THE ROLE OF THE COACTIVATOR SRC-1a IN PROSTATE CANCER CELLS  
T. Ueda, T.S.Y. Kim, N. Bruchovsky, M.D. Sadar

PP-029  DOES CASODEX INDUCE HORMONE-DEPENDENT PROSTATE CANCER CELLS TO BECOME INVASIVE?  
M. Tenniswood, P. Zhan, J. Walker, E. Chun Yu Lee, K. Packman

PP-030  WITHDRAWN

PP-031  KINESIN LIGHT CHAIN EXPRESSION IN SPERMATIDS  
F.A. van der Hoorn, B. Bhullar, R. Oko, A. Junco

PP-032  GERM CELL TRANSPLANTATION IN HR6B KNOCKOUT MICE  
J.T.M. Vreeburg, H.P. Roest, M.P. Ooms, W.M. Baarends, J.A. Grootegoed

PP-033  CHARACTERIZATION OF RAT 100, A UBC4-DEPENDENT UBIQUITIN-PROTEIN LIGASE INDUCED IN GERM CELLS OF THE RAT TESTIS AND SIMILAR TO THE DROSOPHILA HYPERPLASTIC DISCS GENE  

PP-034  CYCLOSPORIN IMPROVES TESTIS GERM CELL SURVIVAL FOLLOWING MOUSE-TO-RAT TRANSPLANTATION  
Z. Zhang, M. Renfree, R.V. Short

PP-035  DNA REPLICATION AND GERM CELL DEATH DURING SPERMATOGENESIS IN THE RABBIT  
J. Blanco-Rodríguez

PP-036  EFFECTS OF HORMONES ON SPERMATOGENESIS IN MEN WITH HISTOLOGICAL DIAGNOSIS OF SPERMATOGENIC ARREST AT THE PRIMARY SPERMATOCYTE (PS) STAGE  
N. Kanakas, N. Sofikitis, H. Kawamura, T. Mantzavinos

PP-037  ORAL ADMINISTRATION OF DOCSAHEXAENOIC ACID CORRECTS A FATTY ACID DEFECT IN IMMATURE GERM CELLS AND EPIDIDYMAL SPERM AND INCREASES SPERM PRODUCTION IN CFTR-/MICE  
M. Ollero, P. Blanco, S.D. Freedman, J.G. Alvarez

PP-038  SPTRX, A NOVEL THIOREDOXIN EXPRESSED DURING MAMMALIAN SPERM TAIL ELONGATION  

PP-039  THE MAJOR SUBACROSOMAL OCCUPANT OF BULL SPERMATOZOA IS A NOVEL HISTONE H2B VARIANT INVOLVED IN ACROSOMAL-NUCLEAR DOCKING DURING SPERMIOGENESIS  
R.B. Aul, R.J. Oko

PP-040  LOCALIZATION OF A SPERM CD52 CARBOHYDRATE EPITOPE DURING EPIDIDYMAL MATURATION, CAPACITATION, AND THE ACROSOME REACTION  
Tuesday, June 19

President's Poster Session

PP-041 POSSIBLE IN VIVO MODULATION OF HUMAN SPERM ACROSOME REACTION
BY A CBG-LIKE PROTEIN
W. Miska, R. Henkel, W-B. Schill, R. Sánchez

PP-042 MAP KINASES IN CYNOMOLGUS MONKEY SPERM HYPERACTIVATION
E.N. Haynes, R.J. Swanson, P.F. Blackmore, M.C. Mahony

PP-043 SEMENOGELIN, THE MAIN PROTEIN OF HUMAN SEMEN COAGULUM, INHIBITS SPERM
CAPACITATION AND INTERFERS WITH THE SUPEROXIDE ANION GENERATED DURING
THIS PROCESS
E. de Lamirande, K. Yoshida, M. Yoshiike, T. Iwamoto, C. Gagnon

PP-044 INHIBITION OF SPERM-OOLEmma INTERACTIONS BY THE EXTRACELLULAR DOMAINS
OF CD9 AND CD81

PP-045 SPERM-ZONA PELLUCIDA INTERACTION INVOLVES A CARBONYL REDUCTASE
ACTIVITY IN HAMSTER
L. Montfort, G. Frenette, R. Sullivan

PP-046 CHIMERIC CONSTRUCTS AND DOMINANT NEGATIVE COMPETITORS DEMONSTRATE THAT
A FUNCTIONAL DOMAIN AND THE C-TERMINUS OF PROSAPOSIN (PSAP) ARE REQUIRED
FOR ITS TRANSPORT TO THE LYSOSOMES
S. Lefrancois, C. Knight, D. Ham, C.R. Morales

PP-047 OVER EXPRESSION OF THE HUMAN Asp567Gly FSH RECEPTOR IN TRANSGENIC MICE
M. Simoni, J. Gromoll, V. Nordhoff, S. Schlatt, L. Foppiani, E. Nieschlag

PP-048 FUNCTIONAL DEVELOPMENT OF THE MARMOSET TESTIS; RELEVANCE TO PROTECTION
OF SPERMATOGENESIS IN CHILDREN TREATED FOR CANCER
R.M. Sharpe, C. Kelar, K. Morris, A. Waring, M. Walker, H. Fraser, P. Saunders, C. McKinnell

PP-049 FOG-1 AND FOG-2: TESTICULAR EXPRESSION AND EFFECT ON GATA-MEDIATED
GENE TRANSCRIPTION.
J.J. Tremblay, N.M. Robert, R.S. Viger

PP-050 ELEVATED 17α HYDROXYPROGESTERONE LEVELS IN INFERTILE PATIENTS WITH VARICOCELE
J. Villemur, H. Scaglia, M. Perco, A. Blanco
P1/2 - 001

MORPHOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL STUDIES ON THE ZONATION OF GOAT EPIDIDYMIS
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This study suggests the division of goat epididymis into at least 8 zones i.e. caput : 4, corpus : 3 and cauda : 1. The main cell types include principal ~, basal ~, narrow ~ and apical ~ cells, besides the migratory cells (lymphocytes, macrophages and clear cells). The principal cells are the main cell types. Along the length of the epididymis, the cytology of principal cells shows interesting variations; basal cells and apical cells also sufficiently differ in their cytology. The number of each cell type varies along the length of the epididymis. Histochemically, the choline containing phospholipids (CPL) are of two types i.e. alkali resistant and alkali labile. Alkali resistant CPL are present moderately in zones I, III and IV with more reaction in infranuclear cytoplasm; zones V and VI show strong reaction for alkali labile CPL; zone VII contains both types of CPL. Contrary to the absence of CPL in zone II, the main CPL in zone VIII was alkali labile. The electrophoretic pattern of the proteins in various zones also shows interesting variations. The minimum proteins bands are in zones III and VIII whereas maximum number of these bands are in zone VI suggesting for the total nine zones by dividing cauda further into two (VIII, IX) zones. It is concluded that multidisciplinary approach is a better tool for the zonation of epididymis.

P1/2 - 002

EPIDIDYAL APPROACHES TO MALE CONTRACEPTION
TG Cooper, Institute of Reproductive Medicine, University of Münster, Germany

The advantage of post-testicular contraception lies in its rapid onset of infertility, its reversible nature and the fast return of fertility once therapy is withdrawn. Seeking such "functional sterility" by an epididymal approach would mimic natural infertility stemming from epididymal dysfunction observed in domestic species and some transgenic mice. Three possible approaches to post-testicular contraception are discussed: those involving arrest of muscular contraction at ejaculation; those modulating the composition of epididymal fluid; and those inhibiting sperm function. Compounds that alter the epididymal transport of spermatozoa aim to enhance transport so that immature sperm are ejaculated or to prevent seminal emission leading to azoospermia. Pregnancies and pre-implantation losses may occur if immature sperm or aged sperm are avoided. The latter problems could be avoided by permitting intermittent sexual activity to relieve epididymal pressure. Few compounds are known that modify the composition of epididymal fluid, although immunococontraception against secreted epididymal proteins is being considered for human use. Several compounds are known that inhibit epididymal sperm metabolism with no side-effects at antifertility doses, but ways to minimise side effects at higher doses required for toxicology studies by targeting the epididymis need to be developed.

P1/2 - 003

CELL-CELL INTERACTIONS IN THE EPIDIDYMIS
D.G. Cyr, INRS-Institut Armand Frappier, Université du Québec, Pointe Claire, Qc.

Cellular interactions represent a dynamic process involving cellular recognition, interaction of structural proteins and intracellular signalling pathways. The luminal environment of the epididymis is highly specialized with specific proteins, ions, pHi etc. required for sperm maturation. Tight junctions between epididymal epithelial cells regulate this luminal environment. Claudins are a family of transmembrane tight junctional proteins. Our studies have shown that claudin-1 is present in epididymal tight junctions as well as laterally between adjacent principal cells and between principal and basal cells. Furthermore, using RT-PCR we have identified mRNA transcripts for Cl-6, Cl-7, Cl-8, Cl-9 and Cl-11 in the adult epididymis, demonstrating the complexity of epididymal tight junctions. Cl-1 mRNA levels increase significantly during the formation of the blood-epididymal barrier in the rat. Furthermore, studies using orchidectomized rats and androgen replacement indicate that the regulation of Cl-1 is different between the initial segment and the other regions of the epididymis. In the initial segment, the cellular localization of Cl-1 is dependent on both androgen and other testicular factors while in other epididymal regions, Cl-1 is not regulated by any testicular factor. To further investigate differences in Cl-1 regulation along the epididymis, proteins were isolated from the different epididymal segments and immunoprecipitated. Results indicate at least six different high molecular weight proteins which associate with Cl-1 in all regions of the epididymis suggesting that other mechanisms are responsible for the regulation of Cl-1 in the initial segment including the relationship between components of adhering junctions and those of tight junctions.

P1/2 - 004

ASSOCIATION OF ZO-1 AND BETA-CATENIN IN THE RAT EPIDIDYMIS
S DeBellefeuille* and DG Cyr, INRS-Institut Armand Frappier, Univ Québec, Pointe Claire, QC.

Adhering junctions play an important role in regulating tight junctions. The relationship between adhering and tight junctions remains, however, to be established. It has been reported that ZO-1, a component of tight junctions, associates with beta-catenin, a component of adhering junctions, during tight junction formation. The object of the study was to characterize the composition of adhering junctions in the epididymis and to determine whether or not ZO-1 and beta-catenin associate during the formation of the blood-epididymal barrier. Homogenates of the different segments of the epididymis from 7 and 91 day old rats were subjected to immunoblotting. These data indicate that at both ages the epididymis contains ZO-1 and three different catenins (beta- and alpha-catenin as well as P120). Immunoprecipitation of beta-catenin from adult epididymis indicates that it is associated with alpha-catenin, P120 and E-cadherin. Interestingly, the phosphorylation pattern of beta-catenin is different in 7 day old rats as there are four different phosphorylation states of this protein. In order to determine if beta-catenin is associated with ZO-1, proteins were isolated from 7, 18 and 91 day old rats and immunoprecipitated with either beta-catenin or ZO-1 in order to compare differences in association prior to, during and following the formation of the blood-epididymal barrier. Results indicate that ZO-1 immunoprecipitates contain beta-catenin at all ages. Likewise ZO-1 was present in all beta-catenin immunoprecipitates. Furthermore, alpha-catenin and P120 were also co-precipitated with ZO-1. These indicate that in the epididymis ZO-1 is part of the adhering junction. Furthermore, differences in beta-catenin phosphorylation in young rats suggest that it may play a role in the intracellular signalling of the adhering junction at this age.
P1/2 - 005

Effect of hyperthyroidism on the morphology of ductus epididymis (scanning electron microscopy study). AG Del Rio, LA Palacios*, AM Blanco, H Ncopomnizada*.

Male Fertility Laboratory, Department of Clinical Biochemistry and Division of Endocrinology, Hospital de Clinicas, University of Buenos Aires, Argentina.

The purpose of this study, using light and scanning electron microscopy (SEM), was to observe the effect of hyperthyroidism on the ultrastructure of the rat epididymis. We performed total thyroidectomy by means of an i.p injection of 270 μCi of 131I per rat. After 30 days, the animals were anesthetized with ip sodium pentobarbital. The testes and epididymis were approached as one unit through a scrotal incision. The epididymis was not separated from the testis in order to maintain the blood supply. Portions of the different sections of epididymis smears in celluloid for SEM were obtained. The smears were fixed in 2.5% glutaraldehyde (1% sodium cacodylate in phosphate buffer, pH 7.2), postfixed with 1% osmium tetroxide, dehydrated in acetone, dried by critical point and sputter-coated with paladium-platinum. The observation and photography were made through a scanning electron microscope Ecom IV Philips.

Morphological and physiological differences were detected in the epididymis of the hyperthyroid animals when compared to the controls. In control animals, the luminal surface of the epididymis showed dense layers of stromecia with protruding apical surfaces of pseudostratiﬁed columnar epithelial cells. Secretory granules were abundant in the cells and in the lumen, which had masses of normal looking healthy sperm with well-deﬁned heads and tails. In hyperthyroid rats, remarkable morphological differences were observed. The stromecia were less dense, chilete, broken or absent in many regions giving it a bad appearance. The epithelial cells and the lumen of the epididymis had scanty secretory granules. The cells boundaries were not clear in some regions showing a smooth appearance to the luminal surface. The lumen had sperm, some of which were fragmented.

Our results conﬁrm that hyperthyroidism causes marked structural changes in the ductus epididymis and could affect sperm maturation and motility.

This research was supported by a grant from the University of Buenos Aires (Argentina) UBA-SECYT 01/TB 10.

P1/2 - 006

SPERM MATURATION IN HUMAN EPIDIDYMIS.


We provide new data indicating that the fertilizing capacity of human sperm, expressed as the ability to undergo the acrosomal reaction (AR), bind to the zona pellucida (ZP) and fuse with the ooplasm [HOP]*, develops during epididymal transit. Spermatozoa were recovered from the caput and cauda regions of epididymides from 5 patients undergoing orchidectomy for prostate carcinoma. Ejaculated donor sperm was used as control. AR was assessed by triple stain after incubation for 18hs in HTF-3.5% HSA. An aliquot was subsequently exposed to 20% human follicular fluid (FF). ZP was studied by the hemizona technique. Fusion with the oolemma was assessed by the hamster oocyte penetration test (HOPT). RESULTS: AR (%) ZP binding HOPT -FF and control % penetration Caput 6.1 4.3 5.8 2.3 Cauda 7.9 19.5 37.3 21.8 Control 11.1 29.3 100 59.2. Our results suggest that epididymal transit induces changes in human spermatozoa that enable capacitation and physiological acrosomal fertilization.

P1/2 - 007

IMMUNOLocalization of the Lipocalin EP17 in the Mouse and Rat Epididymis.

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An androgen-dependant lipocalin, epididymal retinoic acid-binding protein (mE-RABP) is secreted by the principal cells of the mouse distal caput and accumulates in the lumen of the cauda. A novel 17KDa lipocalin (mEP17) resulting from gene duplication of mE-RABP was identified. Its mRNA is regulated by testicular factors and restricted to the initial segment. The mature form of mEP17 was cloned into a prokaryotic vector. The recombinant protein band corresponding to mEP17 was excised from SDS-PAGE gel and rabbits were immunized. IgG were used in Western-blotst and immunohistochemistry of Bouin's fixed tissues. The apparent molecular mass of mEP17 was 22KDa but decreased to 17KDa, its predicted mass, after N-glycanase digestion, suggesting that the protein is glycolestated on one or both of its 2 putative N-glycoylation sites. After 2D electrophoresis, mEP17 appeared as a train of 22KDa spots with a range of pI from 6 to 7.5. An EP17-like antigen was recognized in Western-blots using rat initial segment but not in others species. By immunohistochemistry, mEP17 was localized in the supranuclear region of the principal cells of the initiation segment, in the clear cells of the caput and in the lumen of the mid and distal caput but not in the corpus and the cauda. The staining intensi

P1/2 - 008

EPIDIDYMAL TISSUE SPECIFICITY AND ANDROGEN DEPENDENCE OF RAT EP2. NM Ibrahim*, LG Young, and O Frölich*, Emory University, Atlanta, GA.

In primates, the EP2 gene is expressed specifically in the epididymis in an androgen-dependent manner. Here we investigated the pattern of EP2 expression in the rat, by examining the caput, corpus and cauda regions of epididymis and sixteen other tissues. RT-PCR and northern analyses showed that rat EP2 is expressed predominantly in the proximal caput of the epididymis. We also determined EP2 mRNA expression in proximal epididymides from castrated, sham-operated and efferent duct-ligated rats. In castrated rats, EP2 mRNA decreased to <10% of that in sham-operated rats between day 3 and 4 post-castration, demonstrating the androgen dependence of EP2 expression. Injection of testosterone propionate (TP) on days 0, 1, 2 and 3 post-castration maintained EP2 mRNA levels approximately equal to those in sham-operated rats. Starting at day 4 post-castration, daily injections of TP for 7 days restored EP2 mRNA levels to approximately normal levels. In epididymides ligated unilaterally at the efferent ducts, EP2 mRNA levels were approximately equal to those in the unligated contralateral epididymides or in sham-operated rats, indicating that EP2 expression does not depend on testicular factors. These results indicate for the rat that EP2 is expressed specifically in the proximal caput epididymides and that its expression depends on circulating androgens but not on testicular factors.
P1/2 - 009
FERTILITY OF DIFFERENT STRAINS OF RATS SYMPATHETOMIZED WITH GUANETHIDINE (GUA).
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In a previous work, daily administration of a low dose of GUA produced selective denervation of the male sex accessory organs of Sprague Dawley (SD) rats, resulting in an increased number of sperm in the cauda epididymis and in the epididymal transit time, without effect on sperm production. Using natural mating, no litter was produced by GUA-treated rats, because they could not ejaculate into the uterus. It is known that the sympathetomcy produced by GUA is species-specific. The objective of the present work was to study the fertility of Wistar rats, treated following the same experimental protocol, and compare with the results obtained for the SD. 32 rats were randomly assigned to 2 groups of 16 each: 0 (saline vehicle), and 6.25 mg GUA sulphate/Kg body weight, administered daily for 21 days via i.p. injection. At the end of the treatment half of the rats were housed with virgin females for 4h/day (in pairs), for a maximum of 1 week. The day when sperm were found in the vaginal smear was considered day 0 of gestation. Females were killed on gestation day 20 to evaluate fertility. The other half of animals cohabited for 4 h with a proestrous virgin female. The females were then killed and uterine sperm were enumerated. 50% of the GUA-treated rats produced litters and, although there were sperm ejaculated in the uterus, there was a reduction of 2.3 times compared to the control group. The increase in the seminal vesicle weight and in the epididymal sperm number in the GUA-treated animals confirmed the sympathetomcy, although to a lesser extent than observed in SD rats. The data show the importance of considering the strain of rats when studying the effects of sympathetomcy on the fertility of rats. Financial Support: FAPESP.

P1/2 - 010
ABNORMALITIES IN CELLS OF THE TESTIS AND EPIDIDYMIS IN CATHEPSIN A KNOCKOUT MICE.
N Korun*, A D'Azaro*, L Herms, McGill University, Canada and # St. Jude's Research Hospital, Memphis, USA.
Cathepsin A is a lysosomal enzyme that acts as a protease enzyme and catalyzes for α-neuraminidase and β-galactosidase. These glycoproteins form multi-enzyme complexes, which degrade gangliosides. Cathepsin A is localized in Sertoli and Leydig cells and macrophages, as well as in testis, principal, and basal cells of the epididymis. The present study analyzes cathepsin A deficiency on epithelial cell morphology of the male reproductive tract. Cathepsin A deficient mice (−/−) and wild type mice (+/+ ) were fixed with 5% glutaraldehyde, embedded in Epon, and analyzed by light and electron microscopy. Germ and Sertoli cells of the testes appeared unaltered at all ages examined. However, the interstitial space of both testes and epididymis was filled with vacuolated cells, presumably macrophages. In the epididymis, the knockout mice of 2 months showed no noticeable change in the appearance of the epithelial cells of the proximal initial segment (PIS) as compared to wild type. However, in the distal initial segment (DIS), caput and corpus, principal and clear cells accumulated pale vacuoles, presumably lysosomes. The epididymal cells of the cauda showed only minor vacuolation. At 6 and 10 months of age, the same cell types were affected, including the narrow and basal cells of the PIS. The intensity of vacuolation increased with age. In the knockout mice of all ages, the lumen, though at times partially occluded, still had sperm present. Taken together, the data indicate region-specific abnormalities within cells that express cathepsin A. The lack of cathepsin A may account, therefore, for the abnormal accumulation of undigested metabolites displayed as a plethora of vacuoles within the testis and epididymis in a cell and region-specific manner.

P1/2 - 011
ABNORMALITIES IN CELLS OF THE TESTIS AND EPIDIDYMIS IN ADULT MICE WITH CATHEPSIN A DEFICIENCY.
N Korun*, A D'Azaro*, L Herms, McGill University, Canada and # St. Jude's Hospital, Memphis, USA.
Cathepsin A is a lysosomal enzyme that acts as a protective protein and catalyzes for α-neuraminidase and β-galactosidase. These glycoproteins form multi-enzyme complexes, which degrade gangliosides. Cathepsin A is localized in Sertoli and Leydig cells and macrophages, as well as in testis, principal, and basal cells of the epididymis. The present study analyzes cathepsin A deficiency on epithelial cell morphology of the male reproductive tract. Cathepsin A deficient mice (−/−) and wild type mice (+/+ ) were fixed with 5% glutaraldehyde, embedded in Epon, and analyzed by light and electron microscopy. Germ and Sertoli cells of the testes appeared unaltered at all ages examined. However, the interstitial space of both testis and epididymis was filled with vacuolated cells, presumably macrophages. In the epididymis, the knockout mice of 2 months showed no noticeable change in the appearance of the epithelial cells of the proximal initial segment (PIS) as compared to wild type. However, in the distal initial segment (DIS), caput and corpus, principal and clear cells accumulated pale vacuoles, presumably lysosomes. The epididymal cells of the cauda showed only minor vacuolation. At 6 and 10 months of age, the same cell types were affected, including the narrow and basal cells of the PIS. The intensity of vacuolation increased with age. In the knockout mice of all ages, the lumen, though at times partially occluded, still had sperm present. Taken together, the data indicate region-specific abnormalities within cells that express cathepsin A. The lack of cathepsin A may account, therefore, for the abnormal accumulation of undigested metabolites displayed as a plethora of vacuoles within the testis and epididymis in a cell and region-specific manner.

P1/2 - 012
EXPRESSION OF THE PROTO-ONCOGENE C-ROS ALONG THE EXCURRENT DUCT IN HUMANS.
Christine Légaré, Nancy Verville* and Robert Sullivan, Centre de Recherche en Biologie de la Reproduction, Université Laval.
C-ros is a proto-oncogene coding for a receptor-type protein tyrosine kinase. In situ hybridization studies showed that c-ros was expressed specifically and transiently in the mouse embryonic epithelial cells of the kidney, lung, intestine, and the Wolfian duct. C-ros expression in the adult shows great degree of segregation of expression along the epididymis. Male homologous transgenic c-ros knockout mice show abnormality in epididymal epithelium development resulting in the absence of the initial segment. These males are infertile, but their spermatozoa are able to fertilize in vitro. The anatomy of the human epididymis is unusual when compared to other mammals. The caput epididymis is mostly composed of efferent ducts with an undefined initial segment. In the present study, we have examined the expression pattern of c-ros along the human epididymis by RT PCR and by in situ hybridization. Our results showed that c-ros mRNA was expressed all along the human epididymis. Low levels of c-ros mRNA could be detected in the proximal section of each segment of the epididymis. However, c-ros transcript was highly expressed in the distal section of each region of the epididymis. In situ hybridization revealed that c-ros expression was restricted to the principal cells of the human epididymis. These results showed that c-ros expression in human epididymis differs from that in mice. This study suggests that the pattern of gene expression along the excurrent duct varies from one species to the other. (Supported by MRC-Canada).
P1/2 – 013
SPERM PROGRESSION THROUGH THE EPIDIDYMIS (EPI) IS REGULATED NOT ONLY BY NERVE FIBRES BUT ALSO BY HORMONAL FACTORS, AS OXYTOCIN (OT)
Sperm progression through the epididymis (EPI) is regulated not only by nerve fibres but also by hormonal factors, as oxytocin (OT). Receptors for OT (OTR) are present (Kd=2.2±1.25 nM, Bmax=25.4±13 fmol/mg prot.) in rabbit epi and stimulate contractility (EC50=56±29 nM, Emax=54±5% of maximal NA-induced response). After two months from medical castration with a single administration of the long-acting GnRH analog triptorelin pamidote (2.9 mg/kg, n=9) OT responsiveness was completely abolished and OT gene (RT-PCR and Northern analysis) and protein (Western analysis) expression in epi blunted. Administration of testosterone (TE, 30 mg/kg weekly, n=3) did not restore OT sensitivity. Conversely estradiol valerate (EV, 4 mg/kg weekly, n=3) not only completely restored OT response but even amplified it (EC50=9.2±0.9 nM, Emax =97.7±7%). Accordingly, Northern and Western blot analysis indicates that both OTR gene and protein, down regulated by castration, were completely restored by EV but not by TE. To verify whether endogenous estradiol is involved in regulation of OTR expression, we treated intact rabbits (n=3) for 2 months with an aromatase (ARO) inhibitor, letrozole (1.25 mg/day). Blocking ARO activity completely abolished OT activity. This indicates a new action of estrogen in the male: up regulation of OTR in epi. Because OT induced contractility is blunted (-62.3%±6.7) by an ETA antagonist BQ123 (but not by an ETB), we hypothesized that OT action is partially mediated by ET-1. Experiments conducted in cultured epithelial cells form rabbit epi indicate that, indeed OT dose dependently releases ET-1. Our results demonstrate for the first time a paracrine loop between OT and ET-1 that, under estradiol control, regulates rabbit epididymis activity and probably sperm output.

P1/2 – 015
PROCESSING AND LOCALIZATION OF THE D AND E FORMS OF RAT CRISP-1
KP Roberts, KE Ensrud*, DW Hamilton, University of Minnesota, Minneapolis, MN
The sperm maturation protein Crisp-1 (Protein D/E, AEG) is synthesized by the epididymal epithelium and associates with sperm as they traverse the organ. Crisp-1 is produced in two forms, historically referred to as Proteins D and E, that differ only in the most amino terminal portion of the proteins. Polyclonal antibodies to Crisp-1 localize to the sperm head. The common carboxyl-terminal end of the D and E forms of Crisp-1 is predicted to be highly antigenic compared to the amino-terminal end. Consequently, polyclonal antibodies generated against Crisp-1 do not differentiate between the D and E forms. Previously, we generated a monoclonal antibody specific for the E form of Crisp-1 and another to the common carboxyl-terminal region. With the E-specific antibody we have shown that the E form of Crisp-1 is localized to the sperm tail and is proteolytically processed as it associates with the sperm membrane, losing its antigenic carboxyl-terminal portion. This processing explains the general lack of tail staining using antibodies that recognize the common carboxyl-terminal end of Crisp-1. Here we report the characterization of an antibody generated against a peptide in the amino-terminal half of Crisp-1, in a region of identity between the D and E forms of Crisp-1. As predicted, this antibody recognized both D and E forms of Crisp-1 on western blots of sperm membranes and localized to both the head and the tail of cauda epididymal sperm. Western blot analysis using this antibody in combination with our monoclonal antibodies demonstrated that Protein D is also proteolytically processed as it associates with the sperm plasma membrane. These studies illustrate the processing of both the D and E forms of the Crisp-1 protein and the distribution of these protein forms on the surface of rat sperm. Supported by NIH grant HD-11962.

P1/2 – 014
INTRODUCTION: Variations in lipids diet modifies ultrastructure of the urothelial membrane, the immunitary response, the evolution of cancer and the activity of GGT. The gamma glutamyltranspeptidase is an integral protein of the membrane. The glutathione, its natural substrate, has been used in treatment of dyspermic patients, to partially revert damage in spermatozoa plasmatic membrane due to lipoperoxidative processes which take place in epididymis.
OBJECTIVE: The purpose of the present work is to determine:
1) The enzymatic activity of GGT in epididymis, in supernatant and in pellet of epididymal fluid.
2) The presence of microvesicles (MVs) in epididymal fluid.
MATERIALS AND METHODS: male Balb-C mice were used, feed with a comercial diet or semisynthetic diets supplemented with oleine, corn oil or fish. The activity of GGT was identified in: epididymis, spermatozoa and in fractionated epididymal fluid. The pellet obtained by ultracentrifugation was observed using electron microscope, results were analyzed using a multiple regression analysis. RESULTS: microvesicles (MVs) were observed in the epididymis fluid. Two patterns of behavior were distinguished, one for Corn and Fish and another for Corn and Oleine, showing significant difference (P= 0.0001) in K_v values of epididymal caudal region enzyme. The specific activity increase with age.
CONCLUSIONS: The K_v values modifies changing dietary lipids quality, even so, the K_v and V max values of the enzyme are modified according to mice age.
The presence of microvesicles in ultracentrifugation pellets of epididymal fluid was confirmed using electron microscope.

P1/2 – 016
EVIDENCE THAT HEPARAN SULPHATE IS A NUCLEAR DECONDENSING AGENT OF HUMAN SPERMATOZOA IN VIVO.
Human spermatozoa decondense in vitro upon exposure to heparin and glutathione (GSH). The aim of this study was to evaluate whether heparin decondensing ability is related to molecular structure and to study the decondensing ability of other glycosaminoglycans (GAGS). Capacitated spermatozoa obtained from normospermic semen samples (WHO, 1999) were decondensed in the presence of 46μM heparin + 10μM GSH at 37°C. Aliquots were drawn at 15’, 30’ and 60’ and fixed in 2.5% glutaraldehyde in PBS. Nuclear status was determined under phase contrast: U (unchanged), M (moderately decondensed) and C (grossly decondensed). To analyze the effect of heparin molecular structure, sperm were incubated with GSH + heparin or a chemically modified analogue. Heparin and N-desulfated N-acetylated heparin had similar (n=6, ANOVA+Tukey, NS) decondensing abilities. %N=M+G) = 5±1% and 4±1% at 15’; 19±1% and 16±2% at 30’; 22±2% and 22±3% at 60’. ON desulfated N-acetylated heparin was inactive (p<0.05). Decondensing ability of other GAGS was tested by incubating sperm with GSH + heparin, heparan sulphate (Heps), chondroitin sulphate, dermatan sulphate or hyaluronic acid (HA). Decondensing ability of Heps and heparin were similar (n=5, ANOVA+Tukey, NS) at 15’ (3±1% vs 4±1%); 30’ (15±4% vs 16±4%) and 60’ (18±3% vs 19±3%). Chondroitin, dermatan and HA were inactive (p<0.05). Preliminary data suggest that 10% V/V human follicular fluid (hFF) is equivalent with Heps as nuclear decondensing agent in vitro (%N=M+G) = 10±2% for 1heps and 9±2% for hFF at 60’ (n=2). This study shows that heparin decondensing ability in vitro is associated to its sulphation characteristics and provides strong evidence that 1heps present in the oocyte cumulus complex is, together with GSH, responsible for sperm nuclear decondensation in vivo.
P1/2 – 017

FERTILIZATION POTENTIAL OF SPERMATOZOA IN BLOCKED EPIDIDYMIDES. Schosmans R*, Van der Zwalmen P., Van Holmont Hospital, Vilvoorde, Belgium.

Azoospermic males with excretory infertility can obtained help from the microsurgeon in order to restore the patency of the seminal pathways. However, surgery, has its own limitations and at the present time the MESA technique is the most favored approach. The other known method is the TESA technique. The MESA technique consists of collecting spermatozoa from the epididymis to subsequently inseminate oocytes in an in vitro fertilization (I.V.F.) program. The sperm quality, at different levels of blocked epididymides, was found to be acceptable for oocyte insemination when they are collected at a distance of approximately 5 to 20 mm from the rete testis. In this study, covering 89 cases, we employed the MESA approach to compare to existing TESA values obtained from 100 cases. The obtained pregnancy rate with MESA was not related to the site of sperm collection. The overall clinical pregnancy rate was 39%, but the miscarriage rate was as high as 50%. Thus, our results indicate that the MESA technique results in a higher rate of miscarriages than the TESA technique (14% in 100 cases). In conclusion, our group favor the use of TESA over MESA.

P1/2 – 018

IDENTIFICATION AND SEQUENCE ANALYSIS OF A GENOME FRAGMENT ENCODING A ME-RABP RELATED HUMAN ORTHOLOGUE, hE-RABP

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We have previously identified a murine epididymal retinoic acid binding protein (mE-RABP) synthesized in the principal cells of the mouse mid/distal caput epididymidis and secreted into the lumen. This molecule belongs to the lipocalin protein superfamily which bind small hydrophobic molecules such as retinoids. The mE-RABP gene is localized on the [A3-B] region of mouse chromosome 2, a region homologous to the human chromosomes 10[10p] and 9[9q] and known as a lipocalin family gene rich area. To identify the human orthologue of mE-RABP, immunohistochemistry was performed using anti mE-RABP antibody. Immunostaining revealed a mE-RABP like antigen in human caput epididymidis suggesting the existence of a human mE-RABP orthologue. Using the human genome database of Celera discovery system, we found that a 30 kb genomic fragment from human chromosome 9q contains a human mE-RABP orthologue, hE-RABP. The hE-RABP gene is 5 kb in range and displays seven exons and six introns which is the typical organization observed in lipocalin genes. The deduced cDNA sequence of hE-RABP indicates 26% identity with mE-RABP. The predicted hE-RABP amino acid sequence contains the lipocalin motif G-X-W in exon 2 and T-D-Y in exon 4. These results indicate that the E-RABP gene structure is highly conserved during evolution. (Supported by NIH grant HD36900 and a grant from the Rockfeller Foundation/Ernst Schering Foundation.)

P1/2 – 019

CUBILIN AND LRP-2 EXPRESSION IN RAT EPIDIDYMIS

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Recent studies indicate that cubilin, a receptor for high density lipoproteins, intrinsic factor vitamin B12, and albumin, requires the endocytic activity of the low density lipoprotein receptor-related protein-2 (LRP-2/megalin) to mediate ligand endocytosis. While LRP-2 expression in the male reproductive tract has been reported previously, a detailed study of its expression in relationship to cubilin has not been performed. Here we use immunocytochemistry at the LM and EM level to evaluate the expression of these two receptors in the male reproductive tract. Cubilin expression was observed in the corpus and cauda of the epididymis, but was absent from the testis, initial segment and caput. No cubilin expression was detected in the efferent duct, an area known to express LRP-2. At the EM level, double immunogold labeling was done using 15 nm-gold-labeled LRP-2 antibodies and 10 nm-gold-labeled cubilin antibodies. LRP-2 and cubilin were detected within the same endocytic pits, endocytic vesicles and early endosomes in the principal cells, but absent from lysosomes. These findings are consistent with LRP-2 and cubilin functioning together to mediate ligand endocytosis in the corpus and cauda of the epididymis. Furthermore, the findings indicate that both receptors become endocytosed and both are recycled back to the cell surface. (Supported by CIHR to CRM and NIH NHLBI to WSA)

P1/2 – 020

EXPRESSION OF MOUSE EPIDIDYMAL SPAM1 (PH-20), A SECRETORY PROTEIN, IN VIVO AND IN VITRO. H Zhang*, and PA Martin-DeLeon, Department of Biological Sciences, University of Delaware, Newark, DE.

The Sperm Adhesion Molecule I (SPAM1 or PH-20) is an important sperm surface protein with a hyaluronidase activity and bifunctional roles in mammalian fertilization. Recently we reported that in the mouse SPAM1 is synthesized independently in the testis and the epididymis where it is found in membranous vesicles in the principal cells of the epithelium in all three regions (Deng et al. J Androl 2000; 21:822-832). Here we used mouse epididymal luminal fluid and cultured epididymal epithelial cells to demonstrate that epididymal SPAM1 is a secretary protein. Using a dual environment culture chamber system where corpus or cauda epithelial cells are co-cultured with their corresponding epididymal fibroblasts in medium supplemented with androgens and epidermal growth factor, we show that in 2-4 day cultures SPAM1 can be detected immunocytochemically in the epithelial cells. The protein was also detected by Western analysis in extracts of the cultured cells and in their serum-free conditioned medium, as well as in luminal fluid from fresh caput, corpus and cauda epididymis. Importantly, it was shown to have hyaluronidase activity, using hyaluronic acid substrate gel electrophoresis, and to be expressed in greater quantities in the corpus compared to the cauda and caput. The results not only confirm the previous finding that SPAM1 is synthesized in the epididymis, but extend them by showing that it is secreted in the luminal fluid where it may impact post-testicular maturation and function of sperm. Initial results from transcript analysis indicate that epididymal and testicular SPAM1 may be under different transcriptional regulation.
P1/2 – 021
ROLE OF ZINC IN BUFFALO SPERM CAPACITATION AND ACROSOME REACTION
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Sperm capacitation and acrosome reaction are important events before fertilization. In this study, the role of Zn on these two processes has been studied in buffalo which is a very important dairy animal in India. After discarding seminal plasma, sperms were washed with albumin saline (AS), swirled up and incubated in albumin saline, Sp-TALP (containing BSA, taurine, epinephrine, L-glutamine and sodium pyruvate as energy sources) and Sp-TALP+Zn acetate (100μm). Sample was taken at 0 hr and after hourly duration to check its motility, viability, acrosome reaction and for quantitative estimation of proteins (P), acid phosphatase (ACP) and alkaline phosphatase (AKP). Capacitation started after 1 hr and was maximum at 3hrs in Sp-TALP. In Sp-TALP+Zn acetate medium, the motility decreased significantly at 1hr followed by 100% immotile sperms at 3hr. Acrosome reaction started at 2hrs and its percentage increased with time. Quantity of P decreased after 1 hr and again increased at 4hrs in Sp-TALP while it increased significantly in Sp-TALP+Zn acetate and in AS. Specific activity of ACP decreased non significantly in Sp-TALP while its activity decreased significantly in Sp-TALP+Zn acetate and in AS. However, specific activity of AKP increased significantly after 1hr and then decreased after 4hr in Sp-TALP while its activity decreased significantly after 1hr and then increased after 4hrs in Sp-TALP+Zn acetate and in AS.

P1/2 – 022
ACROSOMAL PROTEINASE ACTIVITY OF HUMAN TESTICULAR SPERM AND EPIDIDYMAL SPERM. Wei-Jie Zhu1, Wei-Bo Liang1, and Jin Qing-Liu1; 1Center for Reproductive Immunology Research; 2Dept of Urology, First Affiliated Hospital of JNUMC, Jinan University, Guangzhou 510632, China.
It is well recognized that acrosomal proteinase (AP) plays an essential role in fertilization. Several studies have demonstrated the AP activity of types of ejaculated sperm. However, in human testicular sperm and epididymal sperm, the AP activity have not been fully understood. The present study was designed to investigate the AP activity of human testicular sperm and epididymal sperm. Testicular sperm was obtained from testicular biopsy tissue(n=7). Epididymal sperm was obtained from men with congenital absence of the vas deferens(n=7). A thin gelatin layer assay was used to determine the AP activity. The results showed that unwashed tesicular sperm and epididymal sperm did not appear clear halos around the sperm head, while washed testicular sperm and epididymal sperm revealed AP activity. The halo diameter of the epididymal sperm group was higher than that of the testicular sperm group (P<0.01). The aging and abnormal epididymal sperm decreased or lost the AP activity. From these results, it indicated that human sperm in testis had its AP activity and obtained further development in epididymas. The fluid of seminiferous tubules and epididymis had the inhibitory effect on AP activity. (Supported by Guangdong NSF No. 980701)

P1/2 – 023
CHOLESTEROL REDISTRIBUTION AND EFFlUX IN CAPACITATING SPERM CELLS.
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Mammalian sperm cells are activated prior to fertilization by high bicarbonate levels in the oviduct which facilitates lipoprotein-mediated cholesterol efflux. The role of bicarbonate and cholesterol acceptors on the cholesterol organization in the sperm plasma membrane were investigated. Bicarbonate induced an albumin-independent change in lipid architecture which was detectable by an increase in merocyanine staining (due to protein kinase A-mediated phospholipid scrambling). The response was limited to a subpopulation of viable sperm cells that were sorted from the non-responding subpopulation by flow cytometry. The responding cells had reduced (18%) cholesterol levels compared to non-responding cells. The subpopulation differences were caused by variable efficiencies in epididymal maturation as judged by cell morphology. Membrane cholesterol organization was observed with filipin, which labelled the entire sperm surface of non-stimulated and non-responding cells, but labelled only the apical surface area of bicarbonate responding cells. Addition of albumin caused cholesterol efflux but only in bicarbonate responding cells that exhibited very little filipin labelling in the sperm head area. Albumin had no effect on other lipid components and no affinity for cholesterol in absence of bicarbonate. Therefore, bicarbonate first induces a lateral redistribution in the low cholesterol containing spermatozoa which in turn facilitates cholesterol extraction by albumin.

P1/2 – 024
Spermatozoa bound to the egg's extracellular matrix undergo the acrosome reaction, wherein acrosomal proteases are activated and released. Recent studies suggested that rat caltrin (r-caltrin), a 6.2 KDa seminal vesicle protein may affect fertilization, in part by inhibiting the activity of sperm serine proteinases (Winница et al.,2000). The aim of this study was to evaluate the effect of r-caltrin upon the activation and activity of human sperm acrosomal proteases. In particular, the effect of the inhibitor upon human proacrosin activation was evaluated. Proteinase activation and amidase activity was evaluated on protein sperm extracts (1mM HCl, pH3) using a colorimetric assay (BAPNA), and the effect of the addition of caltrin (0.005-2μM) was assessed. R-caltrin inhibited total protease activity and activity in a dose dependent manner, reaching a 75% of the effect at 0.2 and 0.75 μM, respectively. Among the acrosomal proteases, proacrosin was affected, as evidenced by changes in the proacrosin intermediates pattern in zymography and Western immunobs when activation was performed in the presence of 10 μM r-caltrin. The effect upon activation was dependent on the concentration of the inhibitor and the time of incubation. An antiserum towards r-caltrin specifically recognised a 12-14 KDa protein band in Western blots of human seminal plasma proteins, suggesting the presence of caltrin-like proteins in humans. This study describes the inhibitory effect of r-caltrin upon activation and activity of acrosomal proteases, in particular, upon human proacrosin activation.
Abstracts – Poster Session 1/2

P1/2 – 025

IMPORTANCE OF CALCIUM DURING BOAR SPERM CAPACITATION.
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Mammalian sperm must undergo a preparation period known as capacitation to become capable of fertilizing oocytes. In many species, capacitation is associated with increased cAMP, which activates protein kinase A, leading to sperm protein tyrosine phosphorylation. The signalling events regulating capacitation are poorly understood, especially in boar sperm. Our laboratory recently identified a 32kDa tyrosine phosphorylated protein that is closely related to capacitation. Our objective is to understand the mechanism regulating the appearance of this 32kDa tyrosine phosphoryphoprotein. Our hypothesis was that a cAMP pathway controls capacitation and the appearance of this protein. SDS-PAGE of sperm proteins and immunoblotting with an antiphosphothreonine antibody showed that a phosphodiesterase inhibitor (caffeine) and protein kinase A inhibitor (1189, n=2) do not affect the appearance of this 32kDa protein. We then tested the hypothesis that calcium-related signalling is important during boar sperm capacitation. Inclusion of EDTA, EGTA, BAPTA-K+, BAPTA-AM (n=2) during capacitation revealed that intracellular and extracellular calcium is required for the appearance of the 32kDa tyrosine phosphoryprotein. Flow cytometry with indo-1-AM demonstrated that intracellular calcium increases markedly during capacitation. Calphostin C, was used to test whether protein kinase C regulates capacitation (n=2), although no effect of this inhibitor is observed on the 32kDa tyrosine phosphoryprotein. These results suggest that cAMP is not as important in boar sperm capacitation as in other species, although both extracellular and intracellular calcium are essential. Our interest will now focus on the implication of calmodulin in capacitation and on the appearance of the 32kDa tyrosine phosphoryprotein.

P1/2 – 026

EXPRESSION OF P450 AROMATASE TRANSCRIPTS IN EJACULATED HUMAN SPERMATOZOA. I. Gaeraud-Denis§, S. Lombard§ and S. Carreau§, §. LUPRES EA 2608, University of Caen and §. Department of Genetic and Reproduction, CHRU Caen - France

The conversion of androgens into estrogens is catalyzed by the cytochrome P450 aromatase (P450arom). Indeed, mRNA coding for aromatase and P450arom activity have been detected in Sertoli and Leydig cells as well as in germ cells of various mammalian testes. Conversely, data on testicular aromatase are scarce in men. It has been shown that human spermatozoa contain not only androgens but also, to a lesser extent, estrogens. Therefore we have studied the ability of human ejaculated spermatozoa to convert androgens into estrogens using two approaches: 1) detection of specific P450arom transcripts and 2) measurement of endogenous estradiol. Total RNA was extracted from individual ejaculates of normospermic patients (n = 14, mean age : 33.6 ± 1.7 years) using a guanidium isothiocyanate-isomylacetic acid method. The presence of 18 and 28s rRNA has been detected on 1.5% agarose gel containing ethidium bromide in 10/14 patients. Three responses have been observed when sperm RNA was used as template in reverse transcription polymerase chain reaction (RT-PCR) with P450arom specific primers: 1) presence of a 293 bp expected PCR product (3/14), 2) detection of a weak signal by UV absorbance (6/14) and 3) no visible staining (5/14). But all samples express transcripts for a housekeeping gene GAPDH. Sequence alignments from PCR products of spermatozoa and granulosa cells with published human P450arom sequence are identical except for some bases unidentified. We have confirmed the presence of P450 arom transcripts by semi-nested PCR in the sperm of patients without a positive P450arom mRNA signal in the first PCR. The same results are observed when spermatozoa were treated or not on Percoll gradient (in order to eliminate round cells). The intracellular estradiol concentrations in human spermatozoa are in the range of 47-222 fmol per ejaculate. These data demonstrate for the first time the presence of P450arom gene specific PCR products likely coding for a biologically active enzyme in ejaculated human spermatozoa.

P1/2 – 027

REGULATION OF AROMATASE GENE EXPRESSION IN ADULT MALE RAT GERM CELLS: EFFECTS OF TGFβ, TNα AND TESTOSTERONE. S. Bourgiba*, M. Benahmed* and S. Carreau*, Biochemistry IRBA, LUPRES EA 2608, Caen France and INSERM U 407 Lyon France

The testicular functions are mainly under gonadotropins control but locally-produced factors such as growth factors, cytokines and steroid hormones are also concerned to optimize the spermatogenesis. The ability of the male gonad to transform reversibly androgens into estrogens is well-known and related to the presence of a microsomal enzymatic complex named aromatase (P450arom). In the rat testis besides Sertoli cells and Leydig cells, we have demonstrated that germ cells have the capacity to produce estrogens but the regulation of the P450arom gene expression is poorly understood (1). The aim of our work was to study the putative effects of TGFβ, TNFα and testosterone on the aromatase gene expression in purified adult rat germ cells. Pachytenes spermatocytes (PS) and round spermatids (RS) have the capacity to produce estrogens, the enzyme activity is 3 fold-higher in haploid germ cells (178 fmol/mg of protein/h) than in PS. With a specific P450arom set of primers we have shown by RT-PCR that germ cells express aromatase transcripts. In addition using a highly specific competitive RT-PCR method (2) we demonstrated that testosterone enhances the expression of P450arom gene in both germ cell populations whereas an opposite effect is observed with TGFβ. Conversely the addition of TNFα enhances the P450arom transcription in PS although an inhibitory effect is observed in RS. Together with testosterone TNFα induces a decrease of the amount of P450arom mRNA in PS and RS. As a consequence our data suggest that not only promoter I but also promoter I4 direct the expression of aromatase mRNA in germ cells. Therefore in rodent testes meiotic and post-meiotic germ cells represent an additional source of estrogens, together with the presence of estrogen receptors on these cells these data likely suggest a putative role of these female hormones in the control of spermatogenesis in rodents. (1) Levallet et al., 1998, Biol Reprod, 58, 919 ; (2) Genisel et al, 2001, J Endocrinol, 168, in press.

P1/2 – 028


The presence of calcium ions (Ca²⁺) is essential for the maintenance of mammalian sperm function in vitro. However, the specific Ca²⁺ requirements for the occurrence of fertilization-related events in the human is still unknown. The objective of the present study was to determine the extracellular Ca²⁺ concentrations required to support human sperm capacitation, acrosome reaction (AR) and interaction with the zona pellucida (ZP). Spermatozoa from normozoospermic donors were incubated at 37°C, 4, 5 % CO₂ in HSM medium/2.6 % BSA, in the presence of different CaCl₂ concentrations (maximum 2.5 mM, control condition) and several sperm parameters were analyzed. Cells incubated for 18 h in HSM containing at least 0.1 mM Ca²⁺ and then exposed to human follicular fluid (FF) in the presence of 2.5 mM CaCl₂ showed AR Inducibility (I = % FF induced AR - % spontaneous AR) values higher than 20 %, similar to that obtained in the control (I = 22 ± 2 %, n = 8). Calcium concentrations > 0.22 mM were sufficient to support the development of hyperactivation and to acquire protein tyrosine phosphorylation levels comparable to the control (n = 4). On the other hand, spermatozoa exposed to FF in the presence of different Ca²⁺ concentrations required at least 0.58 mM of this cation to obtain the maximum AR response (I > 15 %, n ≥ 4). Similar Ca²⁺ requirements were found for sperm binding to homologous ZP, as revealed in the hemizona assay (n ≥ 5). These results have led us to the conclusion that, while 0.22 mM Ca²⁺ allows the occurrence of human sperm capacitation-related events, 0.58 mM Ca²⁺ are needed to support FF-induced AR and sperm-ZP interaction in vitro.
Abstracts – Poster Session 1/2

P1/2 – 029
DIFFERENTIAL EXPRESSION OF PHOSPHOTYROSINE EPITOSES IN MAMMALIAN SPERMATOZOA FROM DIFFERENT REGIONS OF THE CAUDA EPIDIDYMDIS.
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Tyrosine phosphorylation of sperm proteins is associated with capacitation in vitro but the situation during maturation and storage in the epididymis is unclear. We examined this in the hamster cauda epididymidis where sperm acquire full fertilising capacity and are stored prior to ejaculation. Epididymal sperm were recovered from five subregions (1, proximal; 5, distal), immediately smeared onto a slide and methanol-fixed. Alternatively, the cauda epididymidis was kept intact, flash-frozen in N2,(l) and cryosectioned longitudinally. Presence of sperm phosphotyrosine epitopes was determined by immunofluorescence with a specific monoclonal antibody (4G10 clone). In methanol-fixed sperm, no phosphotyrosine epitopes were detected on sperm in the proximal cauda (regions 1 & 2) but were observed over the equatorial segment of 23 ± 5% sperm from region 3 (mean ± SEM). In regions 4 and 5, the majority of sperm displayed this pattern (94 ± 3 & 99 ± 0.5%, respectively). Examination of the cryosections indicated that there was a sharp increase in the proportion of sperm displaying phosphotyrosine epitopes in the region of duct where the luminal contents are expelled at ejaculation. Similar results were also observed in the mouse and rat. Such a distribution of phosphotyrosine epitopes in sperm in the cauda epididymidis has not been noted previously and may be related to the storage of sperm prior to ejaculation and their subsequent capacitation in the female tract.

P1/2 – 030
MULTIPLE TYPES OF PHOSPHODIESTERASE ISOFORMS IN HUMAN SPERMATOZOA. PARTIAL CHARACTERIZATION. L. Lefèvre*, E. de Lamrander*, S. Rybaltkin*, J. A. Beavo* and C. Gagnon. 1Urology Research Lab, McGill Univ, Montreal, Canada 2Dept of Pharmacology, Univ of Washington, Seattle, WA.

cAMP, an important second messenger, plays an important role in the regulation of sperm motility, capacitation and acrosome reaction. cAMP levels are dependent on the activity of two enzymatic processes: the synthesis by adenylyl cyclase and the breakdown by cyclic nucleotide phosphodiesterase (PDE). The hydrolysis of cyclic nucleotides is performed at a rate of 9 to 600-fold faster than its formation suggesting that PDEs play a dominant role in the control of cyclic nucleotide level in spermatozoa. Furthermore, PDE inhibitors stimulate sperm motility and induce capacitation. Our aim was to identify the different types of PDE present in human spermatozoa. Using family-specific PDE inhibitors, it was suggested that type 1 (calcium-caldulin-dependent) and type 4 (cAMP-specific) PDEs are present in spermatozoa but not type 5 (cGMP-specific). The extraction of PDE activity from human spermatozoa is dependent on the presence of Triton X-100; the activity recovered in the supernatant is 75% as compared to 25% in the particulate fraction and is greater for cAMP than cGMP as substrate. We partially purified these enzymes by anion-exchange chromatography followed by gel filtration. PDE elution profiles of Triton X-100 extract showed major and minor peaks of cAMP/cGMP-PDE activities which are activated by calcium and caldulin and inhibited by specific PDE inhibitors such as EHNA (type 2), milrinone (type 3) and rolipram (type 4). Western blot analysis of the fractions using isozyme-specific antibodies for PDE 1A, 1C and 3A confirmed the presence of these proteins of molecular weight of 67, 41 and 97 kDa, respectively. Moreover, immunolocalization indicated that PDE 1A is present in sperm flagellum, suggesting a potential role in motility. Supported by the MRC of Canada and NIH of USA.

P1/2 – 031
ROLE OF TYROSINE KINASE (PTK) AND cAMP-DEPENDENT KINASE (PKA) CROSS-TALK IN THE REGULATION OF HUMAN SPERM MOTILITY. M. Bajpai* and G.F. Doncel, CONRAD Program, Eastern Virginia Medical School, Norfolk, VA 23507.

Involvement of sequential activation of PKA and PTK in the regulation of sperm motility, although suggested, has not been clearly established in human spermatozoa. In the present study, washed spermatozoa were incubated for 6 h under capacitating conditions (Ham/HSA, 37°C, 5% CO2) with or without PTK (Genistein [GEN]), PKA (H89, H8), or broad-spectrum kinase (Stauorospin [STA]) inhibitors. Additionally, swim-up sperm were pre-incubated with the inhibitors and then stimulated with dbcAMP and the phosphodiesterase inhibitor, pentoxifylline [PTX]. Motion parameters [MPs] (CASA), PTK/PKA activity (ELISA), tyrosine phosphorylated proteins [PYPs] (western blot) and PY-immunoreactive sperm (immunofluorescence) were evaluated. GEN inhibited PTK (but not PKA), decreasing number and intensity of PYPs, incidence of PY-sperm and MPs, especially hyperactivation [HA]; H89, which inhibited PAK but not PTK, did not cause a significant alteration of MPs or PY-phosphorylation, although the intensity of some bands was decreased. cAMP/PTX stimulated PAK and PTK activity, increasing PY-phosphorylation and MPs. H89 reversed such enhancement only partially. GEN, conversely, markedly reduced soluble PYPs and MPs. The PTK activity underlying human sperm motility regulation may be directly linked to capacitation-related changes, not necessarily involving upstream PKA activation. Such event, however, exerts a stimulatory effect. PYPs involved in this modulation are mainly present in the soluble fraction of human sperm extracts.

P1/2 – 032
IDENTIFICATION OF SRC FAMILY TYROSINE KINASES IN BULL GERM CELLS BY RT-PCR
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Sperm capacitation is characterized by the activation of several intracellular signal transduction pathways leading to an increase in the phosphotyrosine content of specific sperm proteins. These proteins have been associated with the acrosome reaction, the acquisition of hyperactive motility, and the interactions with the egg's zona pellucida. In order to better understand sperm capacitation, it seems necessary to identify the protein tyrosine kinases implicated in sperm protein tyrosine phosphorylation. Studies of sperm capacitation with different inhibitors suggest the presence of tyrosine kinases from the src family, regrouping 9 enzymes with strong structural and functional homology. We investigated on the presence of members of the src family of tyrosine kinases in bull testis and enriched germ cells population. This was done by the analysis of 415 base pair sequences fragments obtained by RT-PCR using degenerated oligonucleotides corresponding to conserved sequences among the different members of the src kinases family. In bull testis (18 clones), src (59%), hck (44%), yes (6%), lyn (9%) and fyn (1%) were identified. Hck (54%) and yes (32%) were predominant in haploid germ cells (22 clones), whereas only Lyn (13 clones) was identified in primary spermacocytes. These experiments will help to identify the potential src tyrosine kinases present in mature sperm cells, and to determine whether they are involved in sperm capacitation or other functions. This project is supported by NSERC.

VIIth International Congress of Andrology | 89
Abstracts – Poster Session 1/2

P1/2 – 033

cAMP CONCENTRATION INCREASES DURING ACTIVATION
OF MOTILITY IN QUIESCENT CAUDAL EPIDIDYMAL SPERM
FROM THE RAT
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Mammalian sperm are stored in a quiescent state in the cauda epi-
didymis. Motility is activated during ejaculation, however nei-
thor the primary signal nor the signal transduction pathway(s)
involved in motility activation are known. We have demonstrated
that cyclic AMP (cAMP), the phosphodiesterase inhibitor, pentox-
ifylline (PTX) or calcium (Ca2+) activate short-term motility (30-
60 min.) when mixed with caudal epididymal sperm (CES) from the
rat in the absence of dilution. In contrast, a 5-fold dilution in a
physiological solution containing Ca2+ activates motility that
persists for several hours. Since cAMP and Ca2+ have been implicat-
ed in the regulation of sperm motility, we examined the effects
of Ca2+ and dilution with a modified Tyrodes solution (BWW) on
cAMP concentration in quiescent CES. Optimal motility was activat-
ed by dilution in BWW containing Ca2+ and this caused a rapid
increase in cAMP concentration in CES. However, there was no increase in sperm cAMP when Ca2+ was omitted from the BWW
or when CES were diluted with caudal epididymal fluid (CEF) and
under these conditions motility was impaired. A rapid increase in
cAMP also occurred when Ca2+ was added to CES in the absence
of dilution. As a consequence of these studies we hypothesize that
motility activation in quiescent CES from the rat is associated with
a rapid Ca2+-dependent increase in intracellular cAMP.

P1/2 – 034

INVOLVEMENT OF STORE OPERATED CHANNELS IN THE SUSTAINED
COMPONENT OF THE PROGESTERONE-INDUCED [Ca2+]I SIGNAL IN HUMAN
SPERMATOZOA.
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Addition of thapsigargin to human sperm induces an increase in [Ca2+]i largely
due to Ca2+-influx, providing evidence for a calcium store and capacitative calci-
num entry (CCE) (Blackmore, 1993; Rossato et al., 2001). The progesterone (P)-
induced [Ca2+]i response in human sperm is biphasic, consisting of an initial tran-
sient and a subsequent sustained elevation of [Ca2+]i and is dependent upon Ca2+-
influx (Kirkman-Brown et al., 2000). We have used Fura2 spectroscopy to investi-
gate the potential role of CCE in the sustained component of P-induced Ca2+-
influx.

Application of 10μM thapsigargin to capacitated or uncapsulated human sperm
induced a sustained elevation of [Ca2+]i, which was dependent upon [Ca2+]i. After
stimulation with P, application of thapsigargin between 30 and 180 seconds post-
P (during the decay phase of the transient), induced a large, significant and sus-
tained increase in [Ca2+]i (n=6) that was maintained until the completion of the
experiment (avg. 15min). However, when thapsigargin was applied >180s post-P
(during development of the sustained [Ca2+]i signal) it induced a significantly
smaller increase in [Ca2+]i (n=6). Sperm stimulated with thapsigargin, then with P
generated a [Ca2+]i transient of normal amplitude and kinetics (with respect to the
thapsigargin-elevated [Ca2+]i) but displayed a reduced sustained signal.

These results support data for existence of a Ca2+-store and CCE in human sperm
and provide evidence for the role of CCE in the sustained component of the P-
induced [Ca2+]i signal, as proposed previously (Kirkman-Brown et al., 2000).

REFERENCES

P1/2 – 035

FACTORS REGULATING TYROSINE PHOSPHORYLATION OF
MURINE SPERMATOZOA
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A key regulator of a spermatozoa's ability to fertilise an ovum is a
Ca2+-dependent tyrosine phosphorylation signal transduction cascade that is causally involved in the control of capacititation. The
present study investigated the regulation of tyrosine phosphoryla-
tion in mouse spermatozoa and the role of reactive oxygen species
(ROS) in the signalling pathway leading to capacitation. Caudal
epididymal sperm were collected by backflushing, and suspended
in BWW. Tyrosine phosphorylation was monitored by Western
blot and immunocytochemistry. Most of the phosphorylated pro-
teins were localised to the flagellum. Phosphorylation was inhib-
ited dose-dependently by cholesterol sulfate, diphenyllethiodio-
nium and catalase, but not superoxide dismutase. Phosphorylation increased when calcium was omitted from the
ambient medium, and was further stimulated upon addition of
dibutyryl-cAMP. In contrast, omission of glucose from the medi-
un inhibited phosphorylation via mechanisms that could not be
reversed by NADPH supplementation. These data indicate that
tyrosine phosphorylation in murine spermatozoa is inhibited by
the presence of cholesterol and stimulated by calcium depletion.
The generation of reactive oxygen species (ROS), particularly
H2O2 is positively involved in this signal transduction process via
pathways that involve the mediation of flavoproteins but are not
limited by NADPH availability. Further characterisation of the
mechanisms by which cholesterol efflux, intracellular calcium and
ROS interact in controlling tyrosine phosphorylation is central to
our understanding of the capacitation process.

P1/2 – 036

REGULATION OF HUMAN SPERM CAPACITATION AND
PROTEIN TYROSINE PHOSPHORYLATION BY Ca2+-ATPase.
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Capacitation is a maturational process by which the ability of the
spermatozoon to undergo an acrosome reaction and fertilize the egg are
gained. This event is characterized by membranous and biochemical
modifications in the spermatozoon, resulting in an increase in the
phosphotyrosine content of specific sperm proteins. A number of
studies have demonstrated an increase of intracellular free Ca2+
concentration in the spermatozoon from several mammalian species
during capacitation, providing evidence that Ca2+ ions are of
fundamental importance in this process. Evidence of Ca2+-ATPase
activity in sperm head suggests that the pump may be involved
maintaining a low intracellular Ca2+ concentration. The aim of this
project was to study the role of Ca2+-ATPase in human sperm
capacitation and protein tyrosine phosphorylation. Western blot
analysis using anti-phosphotyrosine antibody have shown an increase
in tyrosine phosphorylation of specific proteins when sperm was
incubated with thapsigargin, a Ca2+-ATPase inhibitor. Also, an
increase in the level of A23187-induced acrosome reaction, as a
capacitation indication, was observed using FITC-labeled lectin.
Conversely, ginseng, a Ca2+-ATPase activator, caused a decrease in
the levels of protein tyrosine phosphorylation and sperm acrosome
reaction. These analyses suggest that Ca2+ plays a crucial role in the
capacitation-associated increase in protein phosphorytrosine content and
that Ca2+-ATPase is involved in the control of intracellular Ca2+
concentration. Further studies will help to better understand the
regulation of sperm Ca2+-ATPase and its involvement in human sperm
capacitation. Supported by FCAR(VD), FRSQ(PL) and CIHR (PL).

90 | VIIth International Congress of Andrology
Abstracts – Poster Session 1/2

P1/2 – 037
CASPASES ARE ASSOCIATED WITH HUMAN SPERMATOGENESIS AND APOPTOSIS IN EJACULATED SPERMATOZOA
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Objective The spermatogenesis in human is controlled by apoptosis including activation of caspases (CP). It was the objective to detect CP in fresh spermatozoa of different qualities and in the germinal epithelium of the testis. Methods Caspa Tag™ kits were applied to detect activated CP in spermatozoa of 10 fresh and of 22 cryopreserved semen samples. They were divided into 2 fractions in dependence on the externalization of phosphatidylserine (PS) at the plasma membrane and its specific binding to Annexin V-paramagnetic micro bead (AN-MB). The passage through a magnetic field separated AN-MB-positive from AN-MB-negative spermatozoa. CP 3, 8, 9 were investigated by western blot (WB, n=15 samples). The detection of CP3 human testes was performed by immunohistochemistry (IH, n = 8). Results Activated CP were found in 42.1±6.3% of fresh and in 47.7±5.8% of cryopreserved spermatozoa (meansSEM, p<0.05). The AN-MB-negative, non-apoptotic, spermatozoa showed a significantly lower percentage of cells with activated CP than AN-MB-positive ones: 9.3±2.2% vs. 89.0±2.3 (p<0.0001). Flowcytometric analyses confirmed with 5.3±1.8% vs. 69.6±5.8% the manual results. IH especially localized CP to spermatozytes. Sertoli cells showed a high level of CP in the Sertoli cell only syndromes (SCO). The WB demonstrated active and inactive CP in the ejaculated spermatozoa. Conclusions The results support the role of CP in controlling germ cell development, they were 1. detectable in ejaculated spermatozoa as well as in germinal epithelium of the testis 2. were especially found in early phases of spermatogenesis and in Sertoli cells of SCO 3. activated after externalization of PS at the sperm surface 4. not significantly modified by cryopreservation.

P1/2 – 038
INCIDENCE OF TAIL STRUCTURE DISTORTIONS ASSOCIATED WITH DYSPLASIA OF THE FIBROUS SHEATH IN HUMAN SPERMATOZOA.
V Raue, G Galaverna, S Brugo Olmedo, A Acosta, H Chemes. CECYR and Lab of Testicular Physiology and Pathology, Endocrinology Dept. Childrens Hospital, Buenos Aires, Argentina.
Dysplasia of the Fibrous Sheath (DFS) is a sperm anomaly found in severe asthenozoospermia. Four patients with a complete form and two with an incomplete form of DFS were studied by electron microscopy (EM) and fluorescence microscopy (FM). Microtubules (Mt) and FS were studied using monoclonal antibodies against α- acetylated tubulin and anti FSC1 (the major protein component of the FS) respectively. Mitochondrial Sheaths (MS) were visualized using the vital dye MitoTracker green and chromatin with Hoescht 33258. Progressive motility was 0 % in all 6 patients. Flagellar pathology approached 100% in the complete form and ranged around 65% in the incomplete form. EM examination disclosed grossly hyperplastic FS, abnormal or absent mid pieces and various degrees of axonemal disruption. Phase contrast and FM showed large numbers of spermatozoa with short, rigid, thick and irregular tails. Hyperplastic FS evidenced excess labelling with FSC1. Axonemal Mt were either reduced, absent or discontinuous. Various pathological patterns of MS were found such as absence, scarcity or "necklace" configurations. The IF approach is an excellent technique to study specific organelles in DFS. While ultrastructural studies of thin sections allow an in-depth knowledge of the internal organization of the tail, fluorescence labelling of selected sperm components afford a unique view of the whole flagellum including topographical relationships of various organelles. The combination of these two approaches is essential for a comprehensive understanding of this pathology.

P1/2 – 039
MULTIPLE POINT MUTATIONS IN THE MURINE SPAM1 GENE ARE ASSOCIATED WITH SPERM DYSFUNCTION IN MICE BEARING THE RB(6.16)24U4B OR RB(6.15)1ALD TRANSLOCATION. Y Zheng, XN Deng and PA Martin-DeLeón, Department of Biological Sciences, University of Delaware, Newark, DE.
In mice bearing the Rb(6.16) or Rb(6.15) Robertsonian translocation (Rb), sperm dysfunction associated with the Rb-bearing sperm leads to transmission ratio distortions (TRDs) in heterozygotes. Here we demonstrate with the Sperm Select assay that the Rb-bearing sperm have significantly decreased (P<0.01) rates of penetration of hyaluronic acid and that, on the basis of litter size, there is reduced fertility in the Rb homozygotes. The severity of the TRDs was directly related to the severity in the alteration of expression of Spam1, which maps to proximal mouse chromosome 6 (MMU 6) and encodes a sperm antigen with hyaluronidase activity. Catalytic kinetics studies indicate that reduced Spam1 hyaluronidase activity in the Rb(6.15) mouse results from a qualitative defect, while for Rb(6.16) with the greater TRD both a qualitative and a quantitative deficiency (confirmed by Western analysis) of Spam1 exist. Six point mutations were shown to be clustered in the Spam1 hyaluronic acid-binding domain in Rb(6.15). For Rb(6.16) which has a gross genomic alteration at the Spam1 locus, 11 point mutations are scattered in the 5' and 3' UTRs and the coding region, where one leads to the replacement of a conserved residue. Entrainment of spontaneous Spam1 mutations, due to recombination suppression near the Rb junctions, is proposed as the major underlying defect of the sperm dysfunction.

P1/2 – 040
EXPRESSION OF GM-CSF RECEPTORS IN BOVINE MALE GERM CELLS
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The granulocyte-macrophage colony stimulating factor (GM-CSF) is a pleiotropic cytokine capable of stimulating proliferation, maturation and function of hematopoietic cells. Receptors for this cytokine are composed of two subunits -alpha and beta- and are expressed on myeloid progenitors and mature mononuclear phagocytes, monocytes, eosinophils and neutrophils, as well as in other non hematopoietic cells. We have recently demonstrated that bull spermatozoa express functional GM-CSF receptors that signal for increased glucose and vitamin C uptake [Zambrano et al. (2001). J Cell Biochem. 80:625-634]. In this study, we analyzed the expression of GM-CSF receptors in bovine germ cells and in epididymal and ejaculated spermatozoa. In testicular sections, both receptor subunits -alpha and beta- were immunolocalized along the whole germ line. Expression of mRNAs for both GM-CSF receptor subunits was further analyzed by in situ hybridization using digoxigenin-labeled probes specific for the alpha and beta subunits. In epididymal spermatozoa, the alpha subunit was localized in the acrosome and sperm tail, and the beta subunit was restricted to the tail. As sperm samples were obtained from the caudal towards the tail of epididymis, the immunostaining was less intense and the percentage of spermatozoa with non-stained acrosomes increased. In ejaculated spermatozoa, both subunits presented the same localization as in epididymal spermatozoa but showed different degrees of staining among different batches of samples, which might indicate variability of their reproductive capacity. Our findings suggest that GM-CSF, a typical hematopoietic growth factor, may have an important role in the physiology of male germ cells. (FINanced by FONDECYT 199-0994).
Abstracts – Poster Session 1/2

P1/2 – 041
PRESENCE AND DISTRIBUTION OF ACTIN ANCHORING PROTEINS IN BULL SPERMATOZOA DURING AND FOLLOWING EPIDIDYMAL TRANSIT.
M. Carbalal*, R. Pelletier, S. Yoon*, M. Vitale*. Pathology and Cell Biology, Université de Montréal, Québec, Canada.
Maturation of spermatozoa (SPZ) is accompanied by changes in actin localization and in G-actin/F-actin ratio. To elucidate the mechanisms involved in actin remodelling during maturation in SPZ, we studied the expression and localization of actin anchoring proteins during and following epididymal transit of SPZ in the bull. Expression and localization of talin, radixin and myosin light chain (MLC) were assessed by Western blot and immunofluorescence microscopy. Talin was highly expressed in epididymal SPZ but a low level of expression was observed in ejaculated SPZ. Talin localized to the mitochondrial sheath and principal piece in both epididymal and ejaculated SPZ. Radixin expression was low in epididymal SPZ but high in ejaculated SPZ. In the latter, radixin localized to the equatorial segment. MLC expression was higher in epididymal than in ejaculated SPZ. In epididymal SPZ, MLC localized to the acrosomal membrane and the post-acrosomal region. MLC in ejaculated SPZ localized to the acrosomal membrane and the membranes of the post-acrosomal segment. The present results show that 1) talin, radixin and MLC localized to distinct regions of SPZ, and 2) the distribution of these proteins is modified during SPZ maturation. Changes in the expression and distribution of actin filament-anchoring proteins may modulate SPZ morphology and influence the outcome of capacitation and acrosome reaction. Supported by CORPAQ.

P1/2 – 042
APOPTOSIS: TRANSLLOCATION OF PHOSPHATIDYLSERINE AND NICKED DNA IN BULL SPERM, DETECTED BY FLOW CYTOMETRY, AND THEIR RELATIONSHIPS WITH FERTILITY.
Translocation of phosphatidylserine (PS) from the inner leaflet of plasma membrane to the outer leaflet and the fragmentation of DNA are two hallmarks of cell apoptosis. In this study, sperm apoptosis was detected in fresh and frozen bull semen. Three ejaculates collected from each of five breeding bulls were cryopreserved by standard industry procedures. Annexin V/PI and TUNEL assays were used to detect translocation of PS and nicked DNA respectively, in fresh and frozen-thawed spermatozoa, by flow cytometry. With the Annexin V/PI assay, for PS exposure on the outer leaflet of cell, four types of sperm populations were observed in fresh semen, i.e. 11.9% apoptotic, 17.0% early necrotic, 13.9% necrotic and 57.2% viable. After cryopreservation, the number of apoptotic sperm increased (P<0.01) to 33.3%, and necrotic and viable sperm decreased (P<0.01) to 5.8% and 43.8% respectively, whereas early necrotic sperm remained unchanged (17.1%, P>0.05). With the TUNEL assay, for nicked DNA, the apoptotic sperm differed in fresh and frozen semen (17.2% and 9.9%, respectively; P<0.05). Bull to bull variation was found in all types of spermatozoa (P<0.05), in both fresh and frozen semen. Bull fertility was correlated (r<0.05) with DNA-nicked apoptotic (r=0.90), necrotic (r= 0.94) and viable (r=0.87) sperm in fresh semen. In conclusion, apoptotic sperm are present in fresh bovine semen. Cryopreservation causes a loss of membrane asymmetry by translocating PS to the outer leaflet of sperm plasma membrane. This study suggests the possible relationships of bull fertility with apoptotic, necrotic and viable sperm. Sponsored by NSERC, SEMEX Alliance and OMAFRA.

P1/2 – 043
CAPACITATED AND CHEMOTACTIC RABBIT SPERMATOZOA APPEAR TO BE SHORTLY AVAILABLE AROUND OVULATION.
L. Giojonal, C. Fabro*, M. Eisenbach*, and R. Rovasio*. University of Córdoba, Argentina1; Weizmann Institute, Israel2.
In mammals that ovulate in response to copulation (eg. rabbits) the egg is ovulated after a defined period of time into an oviduct that already contains spermatozoa. Therefore, in such species there is no obvious need to extend the availability time of capacitated/chemotactic spermatozoa for as long a period as in humans. The aim of this study was to examine whether this basic difference between the species is reflected in the timing of capacitation and chemotaxis. For this purpose, the level of capacitated and chemotactic spermatozoa was evaluated along the incubation time. The percentage of capacitated spermatozoa was determined from the difference in the levels of acrosome-reacted spermatozoa prior and subsequent to acrosome-reaction induction with the calcium ionophore A23187, and by means of the fluorescent acrosome marker Pium sativum agglutinin. Chemotaxis assays were performed by videomicroscopy in a Zigmund chemotaxis chamber. As a parameter for chemotaxis we used the quotient ΔX/ΔY, which is the ratio between the distance traveled by the spermatozoa parallel to the attractant gradient and perpendicular to it. There was a high correlation between the levels of capacitated and chemotactic responsive spermatozoa along the incubation period, with a peak at 16 h. The percentages of capacitated and chemotactically responsive spermatozoa were similar at each time point. We demonstrated that in rabbits, like in humans, a subpopulation of spermatozoa can transiently become capacitated, and that this subpopulation responds by chemotaxis to a follicular fluid factor(s). The results further demonstrate that the timing of sperm capacitation and the duration of the capacitated state are tightly dependent on the timing and life span of the ovulated egg.

P1/2 – 044
DIRECTIONALITY-BASED ASSAY FOR SPERM CHEMOTAXIS, INDEPENDENT OF CHEMOKINESIS AND THE SWIMMING PATTERN.
L. Giojonal, M. Eisenbach*, S. Civalero*, A. Frenkel*, A. Chermgorova* and R. Rovasio*. University of Córdoba, Argentina1; Weizmann Institute, Israel2.
Most sperm chemotaxis assays are based on sperm accumulation at the optimal chemotactic concentration. Sperm accumulation, however, can result from processes other than chemotaxis (e.g. trapping). The goal of this study was to find a parameter that reflects only chemotaxis. Since chemotactically responsive cells move a larger average distance in the direction of the chemooattractant gradient than in any other direction or than the distance traveled by non-responsive cells, it seemed that an assay, based on the direction of swimming, could be a perturbation-free assay for chemotaxis. We followed the tracks made by capacitated rabbit and human spermatozoa in a gradient of follicular fluid or an active fraction thereof (demonstrated earlier to be locomotants) in a Zigmund chemotaxis chamber. We then assessed parameters that potentially reflect the swimming directionality of the spermatozoa: the average net distance traveled by the spermatozoa in the direction of the gradient (ΔX), the percentage of cells with ΔX>0, and the percentage of cells with tracks whose projection on the X-axis is larger than the projection on the Y-axis (ΔX/ΔY)>1. All these parameters indeed reflected chemotactic responsiveness. However, only the percentage of cells with ΔX/ΔY>1 yielded a chemotactic signal independent of other perturbations. This conclusion was substantiated with non-capacitated spermatozoa, unable to respond chemotactically, and with leukocytes in a gradient of a synthetic chemotactic peptide.
P1/2 – 045
ANALYSIS OF THE IMPACT OF CAFFEINE ON MEMBRANE INTEGRITY, REDOX RATIO AND GST IN HUMAN EJACULATED SPERM: EFFECTIVENESS OF ANTIOXIDANTS
M. Arabi1,2, R. Anand1, and U. Kanwar1, 1Department of Zoology, Panjab University, Chandigarh-160 014, India. 2Department of Biology, Shahrekord University, Shahrekord - 88181, I.R. Iran.
Oxidative stress (OS) is known to play a major role in the aetiology of disturbed sperm function, malfunction, via mechanisms involving the induction of lipoperoxidation (LPO) to plasma and intracellular membranes. Exposure of human spermatozoa to caffeine (7 & 9 μM), a methylxanthine stimulator, ameliorated the rate of LPO/peroxidative damage, marginally but significant (p < 0.05, 20 & 29% respectively). The LPO, as an index to membrane integrity assessed by the determination of thiobarbituric acid reactive substances (TBA-RS) was shown to be diminished with Glutathione (GluL), Trolox (a water soluble analogue of Vit. E) and Ascorbate (AA) upto 5, 0.02 & 1.44 μM, respectively. Amongst, Trolox in alone and/or combination form proved to be more effectively than GluL & AA to lower the extent of TBA-RS levels under LPO Ferrous drug inducing system. Redox ratio (α 1/OS) also was impaired by caffeine (p < 0.05) but improved by antioxidants, the best was Trolox (107 & 154%). The drug incubated samples were shown an acceleration in the glutathione S-transferase (GST) activity (0 < 0.01, 158 & 167%) which modulated by antioxidants. The relative impact of caffeine was also registered on spermatozoal morphology. These protective roles of antioxidants may be of clinical values in Sperm-oocyte interactions/fertilization programs.

P1/2 – 046
EFFECTS 17αOH-PROGESTERONE, 17β-ESTRADIOL AND RU486 ON HUMAN SPERM FUSION WITH OOCYTES. COMPARISON AND INTERFERENCE WITH THE EFFECT OF PROGESTERONE.
F. Francavilla, R. Romano, C. Pandolfi, R. Santucci, B. Macerola and S. Francesca. Department of Internal Medicine, Andrologic Unit, University of L'Aquila, Italy
This study aimed to determine the effect of the exposure of human sperm to 17αOH-Progesterone (17OH-P), 17β-estradiol (E2) and RU486 on sperm/oocyte fusion, in comparison with that produced by progesterone (P), as well as the possible interference with the effect of P. Materials and methods. The effect of steroids on sperm oocyte fusion was assessed using the hamster egg penetration test (HEPT). Motile sperm suspensions were exposed to steroid from the beginning or at the end of 5 h-capacitation (x 15 min.) and then washed. Results. The addition of P to capacitated sperm produced a significant enhancement of sperm/oocyte fusion, with a dose/response effect. This enhancement was significantly higher than that produced by the exposure to P from the beginning of capacitation. 17OH-P produced a dose/response effect similar to that of equimolar doses of P. E2 did not produce any significant effect when added either before or after capacitation. Moreover, the sperm preincubation with E2 did not interfere with the effect of P added before or after capacitation. The exposure of capacitated sperm to RU486 produced an inhibitory effect and prevented the stimulatory effect of P only at high dose and if not washed before sperm/oocyte incubation. The exposure of oocytes to RU486 followed by washing did not affect sperm/oocyte fusion. Conclusions. The present data support the hypothesis that 1) 17OH-P acts through the same receptor and/or the same traduction pathways of P and 2) RU486 is able to bind the membrane receptor of P, but with very low affinity. No effect of E2 is demonstrable on sperm/oocyte fusion.

P1/2 – 047
IDENTIFICATION OF E, P, AND N CADHERINS IN HUMAN SPERMATOZOA.
Cadherins are a superfamil of transmembrane glycoproteins involved in calcium dependent cell-cell adhesion events. The present study was aimed at examining the expression of E (epithelial), P (placental), and N (neural) cadherins in human testicular and epididymal tissues, and their localization in human ejaculated and capacitated spermatozoa. Presence of cadherin molecules was demonstrated using specific antibodies towards E, P, and N cadherins (Santa Cruz Biotechnology, USA). Their expression in human epididymal and testicular tissues was analyzed by immunohistochemical analysis and in Western immunoblots of total protein homogenates. Localization of cadherin in whole spermatozoa was determined on fixed and permeabilized cells from normozoospermic donors. Anti-E cadherin specifically recognized a protein of Mr 120-127 in Western immunoblots of total epididymal proteins, and stained only epithelial cells in tissue sections. With anti-N cadherin, an specific staining of cells from the seminiferous epithelium was found, and a 121 KDa protein band was immunoreactive in human testicular homogenates. In whole ejaculated and capacitated spermatozoa, a strong staining was observed with all three antibodies, which was mainly confined to the postacrosomal and midpiece regions. The studies describe the expression of cadherins in tissues of the male reproductive tract. In addition, they are the first showing detection of E, P, and N-cadherin proteins in ejaculated and capacitated human spermatozoa, suggesting a role in the sperm-egg interaction process, which is currently under investigation.

P1/2 – 048
ROLE FOR SPERAD?
I. Ilaparuma1, C. A. Allen2, B. Canas3, P. Hessel4, D. Pappin2, J. Stanton2, L.C. Fisher2 and D. P. L. Green2. 1 Department of Anatomy, University of Ruhrhaus, Sri Lanka. 2 Department of Anatomy, University of Otago, New Zealand. 3 Imperial Cancer Research Fund, London. Mammalian sperm fuse with eggs using the equatorial segment. We present evidence that the equatorial segment contains sperad, a putative adhesion protein. 48 kDa G11 protein was purified from guinea pig testis by immunoaffinity chromatography using monoclonal antibody (mAb) G11 and was subjected to amino acid microsequencing by tandem mass spectrometry. Internal amino acid sequence data revealed sequence homology with sperad. A striking feature of the cytoplasmic domain of sperad is the presence of a repetitive proline rich sequence, PQQPEQ, which is unique to sperad and hence to sperm. The cytoplasmic domain of sperad was amplified from a, guinea pig testis cDNA expression library by PCR and tested for its ability to bind mAb G11 by expression in a prokaryotic gene fusion system as fusion with glutathione S-transferase. These experiments confirmed that the G11 epitope is specific for the cytoplasmic domain of sperad. The cytoplasmic domain of sperad could act like other cytoplasmic proline rich regions as an intracellular signalling molecule. Since sperad is exposed to the egg following sperm-egg fusion it could act as an egg activating molecule.
P1/2 - 049
IN VITRO FERTILIZING CHARACTERISTICS OF BOVINE SPERM WITH ABNORMAL MORPHOLOGY
J. Thundathil*, A.D. Barth, and R.I. Mapleton. Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK Canada.
A combination of in vitro fertilization and other sperm function tests were used to determine the fertilizing characteristics of bovine sperm with abnormal morphology. Semen samples containing a high proportion of sperm affected with knobbed acrosomes, pyriform heads, nuclear vacuoles or proximal cytoplasmic droplets were frozen and stored for the study. Sperm affected with knobbed acrosomes, pyriform heads, and nuclear vacuoles had reduced ability to bind to the zona pellucida. However, sperm with proximal droplets did not bind at all. Knobbed sperm underwent spontaneous acrosome reaction immediately after thawing suggesting impaired plasma membrane function and reducing their ability to interact with oocytes. The ability to fuse with the oolemma and undergo nuclear decondensation was also impaired in knobbed sperm. Pyriform sperm appeared to have a reduced ability to initiate cleavage. Vacuolated sperm penetrated the ooplasm, and appeared to participate normally in fertilization with the resulting zygotes developing through to morulae and blastocysts. Normal appearing sperm co-existing in the semen along with high proportions of knobbed sperm or proximal droplets were also functionally deficient, resulting in reduced fertilization and embryo production. Results suggest that impairment in mechanisms of sperm-oocyte interaction is similar among various types of abnormal sperm. Therefore, abnormal morphology may be considered a marker of functional deficiency of normally appearing sperm in a sample. Since co-existing morphologically normal sperm may be functionally impaired, fertility of bulls affected with a high proportion of abnormal sperm may not be greatly improved by increasing the number of spermatozoa.

P1/2 - 050
CHARACTERISATION OF HUMAN SPERM ANTIGENS EXPOSED AFTER THE ACROSOME REACTION THAT MAY PARTICIPATE IN GAMETE FUSION PROCESSES.
N Al-Eisa, RO Ojo and HDM Moore. Department of Molecular Biology & Biotechnology, University of Sheffield, S10 2UH, U.K.
To identify human sperm receptor molecules potentially involved in gamete fusion a panel of monoclonal antibodies were produced against purified human sperm heads demuced of their apical acrosomes. Two mAbs were selected for further investigation based initially on their localisation to the equatorial segment (1.97, ES-1). Immunolocalisation by light and electron microscopy revealed that the 1.97 antigen was localised on the inner acrosomal membrane(IAM) and equatorial segment (ES). ES-1 antigen was restricted to the ES after induction of the acrosome reaction (AR). In the tests both antigens were expressed on developing spermatids and testicular spermatozoa. Under non-reducing conditions, immunoblotting with mAb 1.97 detected a characteristic ladder of protein bands (45-29 kDa) in human ejaculated sperm and purified heads but not in spleen, liver, brain, ovary. The pI of the 1.97 antigen was 9.8. Under reducing conditions mAb ES-1 detected a single band at 40 kDa in sperm but not other human tissues. Both mAbs reduced the binding of capacitated human sperm to cryopreserved zona-intact human oocytes. Human sperm binding to the oolemma of zona-free hamster oocytes was also reduced after treatment with either mAb at the highest concentration. However, sperm-egg fusion was reduced significantly with mAb ES-1 and antigen was displayed on sperm bound to the oolemma. In summary, the study has identified two candidate antigens (1.97, ES-1) of the human sperm acrosome that are exposed only after the AR and may contribute to the process of sperm-egg fusion.

P1/2 - 051
FINE MAPPING AND CHARACTERIZATION OF THE Stop1d (SPERM-t COMPLEX-OOCYTE PENETRATION 1-DISTAL) LOCUS IN THE t HAPLOTYPY REGION OF Mus musculus
AA Redkar, L Hui*, P Olds-Clarke, and SH Pilder, Dept. of Anatomy & Cell Biology, Temple University School of Medicine, Philadelphia, PA.
1 Haplotypes (t) are naturally occurring variants of proximal Mus musculus wildtype (+) chromosome (Chr) 17 that historically have served as excellent models for studying the molecular basis of sperm function in fertilization. Male mice homozygous for t (tt) are sterile, and express the "stop" phenotype, an inability of sperm to penetrate the oolemma. Because high resolution mapping of genes is difficult in t haplotypes, we previously used male mice carrying heterospecific combinations of the t region (S+ Chr 17 homologs) that produced the "stop" phenotype in males also carrying a single t haplotype (S+/t) to identify two putatively interacting loci in the distal part of the region (Stop1p and Stop1d; Redkar et al, 1998, Mamm Genome, 9: 825-830). However, the Stop1d locus was resolved to only a moderate resolution, and its contribution to the penetration phenotype (binding or fusion) was unclear. We have since employed simple sequence length polymorphism (SSLP) analysis of new S+ Chr 17 homologs to localize Stop1d between 19.2-22.9 CM, a region containing numerous testis- or epididymis-expressed genes, including two possible candidates, Ag1 and Tph1. Furthermore, our data show that Stop1d expresses a strong sperm-oolemma binding phenotype, as determined by analyzing sperm from heterozygotes carrying t and either partial t haplotypes (Redkar et al., 2000, Devel Biol, 226: 267-280) or S+ Chr 17 homologs.

P1/2 - 052
ANALYSIS OF THE ROLE OF CARBOHYDRATES AND DISULFIDE BONDS IN THE BIOLOGICAL ACTIVITY OF EPIDIDYMAL PROTEIN "DE".
DA Ellerman*, MM Morgenfeld*, VG Da Ros*, D Busso*, DJ Cohen* and PS Cuanciu. Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina.
Rat epididymal protein DE participates in gamete fusion through complementary sites on the egg surface. DE (32kDa) contains 10% carbohydrates (CH) and 16 cysteines involved in disulfide bonds (S-S). To investigate the relationship between the structure and function of the protein, we have expressed DE in a bacterial system (recDE) and examined the relevance of CH and S-S in the activity of the protein. RecDE, fused to maltose binding protein (MBP), was used in vitro to test DE and its disulfide linkages (IVF). The presence of recDE during gamete co- incubulation produced, like native DE (nDE), a significant (p<0.01) inhibition in the % of fertilized oocytes compared to controls (recDE: 24%, nDE: 11%, MBP: 62%, no protein: 72%). In order to study whether the lower inhibitory activity of recDE (ED50: 10μM) compared to nDE (ED50: 3.2μM) was due to its lack of CH, nDE was treated with PNGaseF and used in IVF. The %f in the presence of deglycosylated DE (14 %) was not significantly different from that corresponding to nDE. To examine the formation of S-S in recDE, free-SH groups were labelled with biotin-maleimide and visualized by avidin-peroxidase. The results showing no labelled bands in nDE and a clear signal in recDE indicated a deficient formation of S-S in the recombinant protein. The relevance of S-S for the activity of DE was finally indicated by the finding that dithiothreitol-reduced and alkylated nDE produced a significantly lower inhibition of egg penetration (%f: 51%) compared to untreated nDE (%f: 16%). Together, these results indicate that while the CH would not be involved in the activity of DE, the S-S would play an important role in the activity of the protein.
P1/2 - 053

PT32, A POTENTIAL CANDIDATE FOR A SPERM BORNE EGG ACTIVATING FACTOR.

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The perinuclear theca (PT) is a condensed cytosolic structure surrounding the eutherian sperm nucleus and is implicated in oocyte activation during fertilization. In search of potential activating proteins within the PT, bull testicular CDNA libraries were immunoscreened with anti-sera raised against the PT. One of the clones identified is a novel 32-kDa protein (PT32) which has sequence similarities to WBP2, a protein involved in the Src tyrosine kinase pathway. Antibodies raised against recombinant PT32 localized it exclusively to the postacrosomal sheath of the sperm head and showed it to originate in elongated spermatids. Oocyte microinjection of either PT extract or recombinant PT32 showed a significant activation rate over the sham injected controls and immediately triggered tyrosine kinase foci within the ooplasm, followed by the pronucleus formation and zygotic cleavage. Supporting its role as an oocyte activating factor, we also found PT32 to be absent in globozoospermic spermatozoa and round spermatids which normally fail to activate the egg during ICSI and ROSI respectively. In addition, PT32 is found in bull and primate sperm freeze-thaw extracts known for interspecies egg-activation ability on microinjection. Thus based on its possible involvement in the Src tyrosine kinase signaling cascade, its strategic localization in the PT and its ability to activate the metaphase II oocyte, PT32 could be a key factor, or a cofactor, in unveiling the oocyte activation pathway (supported by NIH, USDA and CIHR).

P1/2 - 055

MODULATION OF HUMAN SPERM CAPACITATION BY ENDOMETRIAL CELLS. J. Laflamme*, A. Akoum* and P. Leclerc, Centre de recherche du CHUQ, Pavillon St-François d'Assise, Centre de Recherche en Biologie de la Reproduction (CRBR), Université Laval, Québec, Canada.

To achieve fertilization, mammalian spermatozoa must undergo several biochemical and membranous changes commonly called capacitation. A large part of this process takes place in the female reproductive tract, where the spermatozoa is in contact with different cell types and their secretory products. Some studies have previously shown that endometrial cells can exert a positive effect on human sperm as far as it concerns motility. The aim of this project is to evaluate the effect of endometrial cell and their secretory products on human sperm capacitation and protein tyrosine phosphorylation. In order to mimic the in vivo conditions, freshly ejaculated human sperm were washed by Percoll gradient and incubated with conditioned medium (CM) from epithelial or stromal cells and from frozen-thawed stromal cells. The capacitation process was assessed using the calcium ionophore A23187-induced acrosome reaction and the percentage of acrosome reaction was evaluated by PSA-FITC staining. The effect of endometrial cells on sperm protein tyrosine phosphorylation was assessed by Western blot using an anti-phosphotyrosine antibody. Our results suggest that CM from epithelial cells capacitate human sperm better than CM from stromal cells (fresh or frozen-thawed). However, frozen-thawed stromal cells seem to be better inducer than fresh stromal cells. Our results also suggest that epithelial cells have a greater effect than stromal cells on sperm protein tyrosine phosphorylation, and that frozen-thawed stromal cells can improve this phosphorylation better than fresh stromal cells. All these results are physiologically relevant since epithelial cells are the ones that are normally in contact with spermatozoa. Further studies will help to better understand endometrial cells involvement in human sperm capacitation. This research is supported by the CIHR.

P1/2 - 056

EFFECTS OF TESTOSTERONE ON BEHAVIOR AND MOOD IN EUGONADAL AND HYPOGONADAL MEN.

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The relationship between testosterone (T) and sexual behavior is well known. However, less is known about the non-sexual effects of T. This study aimed to investigate the effects of supraphysiological levels of testosterone (T) on behavior (e.g. aggression) and cognitive functions (e.g. visuospatial ability) in young men. Thirty healthy eugonadal men with partners and 8 hypogonadal men participated in a single-blind, placebo-controlled study. Eugonadal subjects were randomised into two groups to receive: 1) 200 mg T enantate, IM, weekly for 8 weeks raising T levels into the supraphysiological range (active group) or 2) 200 mg saline, IM, weekly for 8 weeks (placebo group). The hypogonadal group received a physiological regime of 200 mg T enantate, IM, bi-weekly for 8 weeks. All completed a range of behavioral measures and cognitive tests at baseline, week 4 and week 8. No statistically significant changes in self- or partner-reported aggression levels, self-esteem or assertiveness were found in response to T treatment. Trait Impulsivity was generally found to be only the significant predictor of Total Aggression. The hypogonadal group reported significantly higher levels of hostility, anger and irritability at all time points compared to the active and placebo groups. They also reported significant reductions in negative mood scores (e.g. tension-anxiety) during treatment. Verbal fluency increased significantly, whilst scores on the visuospatial test decreased in the active group compared to that of the placebo group by week 4. No significant changes were found for the other tests. Our findings do not support the hypothesis that supraphysiological levels of T lead to increased aggression or mood disturbances. Elevated negative behavioural parameters (e.g. verbal aggression, hostility & anger) were consistently observed in the hypogonadal group with and without treatment. Increased T has a differential effect on cognitive function in normal men, inhibiting spatial abilities while improving verbal fluency. These findings may be relevant for T replacement therapy and hormonal male contraception.
P1/2 – 057
ANDROGEN FUNCTION OF ADRENALES AND TESTES IN PATIENTS WITH HYPERGONADOTROPHIC HYPOGONADISM
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The adrenal androgen function in men with hypergonadotrophic hypogonadism (HH) is a subject of big clinical interest. The aim of the present study was to evaluate androgen function of adrenalectomized patients with HH. Thirty nine men from 24 to 48 years (mean age 32.5 + 6.4 years) with primary testicular disorders were studied. Hormonal tests included basal levels of LH, FSH, prolactin (Prl), progesterone (P), 17α-hydroxyprogesterone (17-OHP), androstenedione (A), androstenedione-S, testosterone (T), androstenedione-S, testosterone (T), 17β-estradiol (E2). Medium LH levels were 19.0 + 9.5 U/L, FSH - 25.2 + 10.3 U/L, Prl - 352.2 + 166.2 U/L, T - 8.9 + 3.1 nmol/L, P - 2.5 + 1.2 nmol/L, 17-OHP - 7.0 + 3.5 nmol/L, F - 474.8 + 190.2 nmol/L, DHEA - 26.0 + 11.9 nmol/L, DHEA-S - 3182.1 + 1323.7 nmol/L, E2 - 176.4 + 98.9 pg/mL. Testosterone levels correlated positively with Prl (r = 0.44, p = 0.019); Prl levels with E2 (r = 0.39, p = 0.040). LH levels correlated negatively with T (r = -0.51, p = 0.005). We did not find a correlation between basic adrenal and testicular androgens in patients with HH. The possible explanation of this fact could be that androgenoses in both adrenals and testes have different independent regulatory mechanisms.

P1/2 – 058
EFFECT OF FOOD ON THE ORAL BIOAVAILABILITY OF A NEW ANDRIOL® FORMULATION.
Introduction: Testosterone undecanoate (Org 538), a fatty ester of the natural hormone testosterone, is used for clinical situations of testosterone deficiency. The compound is designed to deliver testosterone to the systemic circulation via the intestinal lymphatics, thereby circumventing first pass inactivation by the liver. Currently Org 538 is dissolved in oleic acid and marketed as Andriol®. To allow more convenient storage conditions a new formulation was developed in which oleic acid is replaced by castor oil.
Method: To determine the effect of food on the oral bioavailability of this new formulation an open-label, two period, cross-over trial in 16 healthy postmenopausal females was performed. Org 538 (80 mg) was administered with and without a normal breakfast in a randomized order and a wash-out period of 7 days between treatments. Blood samples were collected regularly until 24 hours after dosing to determine serum concentrations of testosterone using a GC-MS assay.
Results: Food had a major effect on the bioavailability of Org 538. The maximum serum testosterone concentration (Cmax) occurred at 5.0 h after dosing and was 20 times higher when Org 538 was administered to fed rather than fasted subjects. A 13-fold increase in area under the concentration versus time curve (AUC) of testosterone was shown in the fasted state. This increase is likely to be due to increased solubilization and facilitation of lymphatic transport of Org 538 as a result of lipid digestion and absorption of the co-administered food.
Conclusion: Intake of the new Org 538 formulation with food significantly enhances the bioavailability of testosterone relative to the fasted state. As a consequence it is concluded that Andriol® must be taken with meals.

P1/2 – 059
DOSE PROPORTIONALITY OF A NEW ANDRIOL® FORMULATION.
Introduction: Testosterone undecanoate (Org 538) is used in clinical situations of testosterone deficiency. Currently Org 538 is dissolved in oleic acid and marketed as Andriol®. To allow more convenient storage conditions, a new formulation was developed.
Objective: To assess the dose proportionality of three different doses of this new oral Org 538 formulation, based on testosterone concentrations.
Methods: An open-label, group comparative, parallel design study was conducted in healthy female volunteers of non-childbearing potential. Subjects were randomized to receive one of the following single-day treatments: 20, 40 or 80 mg of Org 538 in the morning and a same dose in the evening. Doses were taken directly after intake of a normal meal. Serial blood samples were collected until 48 hours after the morning dose to determine serum testosterone concentrations using a GC-MS assay. Safety was assessed according to standard techniques.
Results: 45 subjects completed the study. Following administration of Org 538 in the morning testosterone concentrations rose until reaching a maximum value (Cmax) on average at 6 h after dosing (mean Cmax = 1.81, 3.86 and 7.68 ng/mL for 20, 40 and 80 mg resp.). Concentrations subsequently declined until administration of the evening dose 12 hours later. Tests on pharmacokinetic parameters of testosterone (Cmax and AUC calculated both with and without correction for baseline testosterone) indicated dose proportionality within the dose range studied. Few, mild adverse events were observed.
Conclusion: Oral administration of the new Andriol® formulation leads to a dose proportionate increase in testosterone within the dose range studied (20 to 80 mg). The new Andriol® formulation was well tolerated without showing any dose dependent adverse events.

P1/2 – 060
THE U.S. ANTIDOPING (USADA) RESEARCH SUMMIT SUMMARY. JL Fournroy, Uniformed Services University of Health Sciences, Bethesda, Md. 20814
The United States Antidoping Agency (USADA) was formed to bring transparency and oversee all doping issues for the U.S. Olympic Committee after October 1, 2000. USADA is in charge of testing, research, and education with an aim of improving the ethics and integrity of all sports. USADA brought together forty experts to identify the scientific issues and prioritize future research needs. The breadth of this scientific summit covered a wide range of doping problems, e.g., androgens/anabolic steroids, dietary supplements, erythropoietin, growth hormones and related peptides as well as important issues of addiction and ethical concerns related to all future research. In the United States androgens/anabolic steroids and dietary supplements with androgenic components continue to play an important role in doping. Although the carbon isotope (CIR) and the 3:1 ratio (testosterone:epitestosterone) tests appear promising, continuing studies will better confirm and identify possible outliers. Androgenic dietary supplements, e.g., nandrolone, androstenedione, and andriol, are prohibited by International rules but continue to be problems in the U.S. due to lack of manufacturing oversight and availability. This presentation will summarize the identified priorities of the Research Summit.
P1/2 – 061
L-CARNITINE AND FERTILITY IN RAMS
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Supplementation of L-Carnitine (Carniking, Lonza Ltd., Basle, Switzerland) has a positive effect in improving the general energy and feed metabolism, thus influencing the reproductive processes in male animal. L-Carnitine was supplemented for 2 weeks on a daily basis, to 2 groups of Rahmani rams (group 1 normal fertile rams, and group 2 sub fertile rams). Semen and serum samples were collected from all rams before (control) and after L-Carnitine supplementation for assessment of semen quality. LH and testosterone concentration. An increase in values of the studied semen parameter was observed in all rams. LH and testosterone concentration showed a significant increase in sub fertile rams together with a significant improvement in semen quality (sperm motility, % of a live spermatozoa and sperm cells concentration). L-Carnitine serves as a physiologically important energy substrate, supporting the synthesis and transport of ATP. It could be concluded that L-Carnitine supplementation has improved the fertility of normal and sub fertile rams. Increasing the sperm yield and improving the semen quality, especially in sub fertile rams.

P1/2 – 062
NO DECLINE IN SPERM QUALITY IN A COMMUNITY BASED STUDY OF MEN UNDERGOING VASECTOMY WITHIN 20 YEARS.
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Introduction: In the last few years there is a discussion about the decrease of sperm quality in the industrialized countries. Many environmental and lifestyle factors were evaluated for their risks. Many studies have been published, but a big problem remains the selection of the cohort of men (e.g. infertile men, sperm donors etc.). The aim of this study was to assess the sperm quality of proven fertile men, who underwent vasectomy for family planning reasons. Material and Methods: Between 1980 and 2000, 1257 patients were vasectomized in our department to provide permanent birth control for men. We applied the No-Scalpel vasectomy method. The mean age of the patients was 36.6 years, ranging from 22 to 61 years. Prior to vasectomy, a sperm count was performed in every patient. For technical reasons, only the sperm concentrations were evaluated. Results: In this period of time, there was no significant decrease in sperm concentrations matching every single year. Moreover the sperm count between 1990 and 2000 was significantly higher compared to the years 1980-1989 (75.6 Mio/ml versus 65.7 Mio/ml). Interestingly, there were also significant seasonal changes in sperm counts, with the highest concentrations in wintertime. Discussion: Changing sperm qualities seem to be more a regional than a global problem. It is also important to keep in mind, what cohort of men were evaluated in various studies. Men from infertility clinics do not seem to be the proper candidates for trends in sperm quality. In men with proven paternity, no changes in sperm concentrations could be detected.

P1/2 – 063
PREPARATION OF A MORPHOMETRIC STANDARD FOR HUMAN SPERM
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INTRODUCTION: The WHO laboratory manual defined the normal morphology of human sperm head as an oval-shape with regular outline, but it did not refer to the relationships among the morphology and physiological functions. To measure human sperm morphology using a computer assisted image analyzer (CAIA), it is essential to establish the morphometric standard of human sperm. The present study defined functionally normal sperm as those with progressive motility, with the apparent density of more than 1.13 g/ml and capable of inducing the acrosome reaction, in vitro. The digital image of the purified sperm was assessed with CAIA. MATERIALS AND METHODS: Mature sperm with an apparent density of more than 1.13 g/ml was condensed using the continuous-step density gradient centrifugation in the isotonic 99% Percoll, then progressively motile sperm was separated by the swim down procedure. The acrosome reacted sperm was separated by cell affinity chromatography on concanavalin A conjugated Sepharose. The purified sperm was then stained with 0.03% Rose Bengal. RESULTS: After purification, the rates of motile sperm and acrosome reacted sperm were 92±/2.6% and 86±/-4.1%, respectively (n=3). The head morphology of purified sperm was homogeneous in the oval shape. CONCLUSION: The purified sperm might be available for the morphometric standard for CAIA.

P1/2 – 064
STUNTED TAIL SPERM DEFECT: AN ULTRASTRUCTURAL STUDY OF AN ATYPICAL CASE
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Case Report: A 29-year-old man with four years of primary infertility. He had not history of significant illness and in his family do not exist any ciliar respiratory pathology nor male infertility. Physical examination of the patient showed no pathological findings. The analyses of four semen samples showed: sperm count 67-83 10⁹/ml, and 0% motility. The morphological analysis showed mainly tail disturbances: absence of flagellum: 14-16%; short tail spermatozoa: 45-64%; coiled tails: 12-17%; and an abnormal proportion of spermatids and spermatocytes. Normal spermatozoa were found in a 11-16%. Endocrine profile was found within the normal range. Testicular biopsy revealed impaired spermatogenesis. Scanning electron microscopy revealed sperm heads with intact nuclei and acrosomal regions. To our surprise, the 12% of the stunted tails evaluated had biflagellate arrangement while the other ones had unflagellate tail. Discussion: The our patient's sperm showed some, but not all the defects described for Baccetti for éstump tail syndrome. The spermatozoa showed a complete immobility and most of them had a reduced tail. However, the number of spermatozoa was not reduced and the reduced length of the tail do not affect the total sperm population. Besides, uniflagellate and biflagellate stunted tails were found, in opposite to èstump tail syndrome characterized by uniflagellate spermatozo. Our case confirm the testicular dysfunction of spermiogenesis as the origin of these kind of tail abnormalities. Sperm and cilia disturbances are often reported to be associated. In opposite, our patient had no familial history, neither others cilia disturbance, nor male infertility. To our knowledge, this is the first human sperm in which coexist uniflægelate and biflagellate stunted tails.
P1/2 - 065
EXTERNAL QUALITY ASSESSMENT OF HUMAN SPERM MORPHOLOGY DURING A WORKSHOP INVOLVING 62 PARTICIPANTS
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The aim of the present study was the assessment of the variability in the evaluation of human sperm morphology. Sixty-two technicians and biologists attended the same workshop to classify 100 spermatozoa with numerous anomalies from a single Shorr-stained sample. The high-resolution images of the spermatozoa were captured from a video microscope and directly shown on a large screen. The sperm were further classified by two trained biologists (experts) from a video recording. David's classification was used. It distinguishes normal sperm, fifteen abnormalities of the head, midpiece and tail and offers the possibility of calculating the mean number of anomalies per abnormal sperm (MAI; WHO, 1999). All the data were analysed by accounting for the experience of the participants from the definition of four groups of participants: 1) the entire group, 2) a group of participants with at least two years of practice and reading more than 620 slides per year (n = 11), 3) a subgroup from this last group for whom most results were very close to the expert's results (n = 5) and, 4) a group of inexperienced participants for whom most results were also very close to the expert's results (n = 6). Coefficients of variation were respectively as follows: 41% vs 33% vs 15% vs 26% for the percentage of normal sperm, 12% vs 11% vs 6% vs 3% for the MAI, or 26% vs 12% vs 7% vs 17% for the number of abnormal acrosomes. These data indicated that intensive training and experience reduce the inter-observer variability in sperm morphology assessment. However, this is probably not the only factor involved since some participants with recent or episodic experience had satisfactory results suggesting that a strong visual perception could also contribute to lower variability.

P1/2 - 066
LOWER PERCENTAGE OF SPERM WITH NORMAL MORPHOLOGY (NM) USING STRICT CRITERIA IS NOT ASSOCIATED WITH LOWER PREGNANCY RATES (PRs) FOLLOWING INTRAUTERINE INSEMINATION (IUI).

There are many studies that suggest that sperm with ≤ 4% NM using strict criteria are subfertile and in vitro fertilization with intracytoplasmic sperm injection (ICSI) may be needed. However, not all studies agree on the clinical importance of the use of NM. In fact, we previously did not find a significantly lower PR following intercourse even with NM ≤ 4% and motile density <10 × 10⁶/mL. The study presented herein evaluated the efficacy of IUI according to NM using strict criteria. The clinical PRs for first IUI cycles were 30.8% (28/91) for 0-4% normal forms, 26.5% (71/268) for range of 5-14% and 20.8% (11/53) for >14%. The amount of motile sperm (x10⁶) available initially was positively correlated with NM in the initial specimen (18.7 for 0-4%, 36.0 for 5-14%, and 79.5 for >14%). Positive correlation was also found with total motile sperm used for IUI (3.12 vs 7.6 vs 16.6, respectively). Thus, the group with the lowest NM and the least number of motile sperm achieved PRs equal or better than groups with higher NM and greater motile sperm concentrations. This study corroborates previous data with intercourse only suggesting that sperm with NM ≤ 4% using strict criteria are not necessarily associated with lower fecundity. These data certainly suggest that IUI should be attempted first when asthenozoospermia is present rather than immediately proceeding to IVF with ICSI.

P1/2 - 067
EVALUATION OF THE HYPO-Osmotic SWELLING TEST IN RELATION WITH SPERM CONCENTRATION AND MOTILITY

We sought to correlate seminal parameters as such sperm concentration and motility with the hypo-osmotic swelling test in samples from sterile and normal donors. Medical records from 215 patients were assessed between January to July 2000. All semen analysis were evaluated manually and with the CASA according the World Health Organization. Results of the hypo-osmotic swelling test (HOS) higher than 60% were considered normal. The median results of sperm concentration, motility and HOS were 77.5 ± 70.30 million/mL, 48.8 ± 18.8%, and 62 ± 0.13, respectively. In 129 patients, the HOS median result was normal (70 ± 0.06) and in 86 patients was abnormal (49 ± 0.09). In patients with a normal HOS test, the median sperm concentration was 100 ± 68 million/mL and the median sperm motility was 58.9 ± 12.8%. In patients with an abnormal HOS test, the median sperm concentration was 44.6 ± 58.9 million/mL and the sperm motility was 34.2 ± 16. In patients with normal sperm concentration (62 ± 8.4) the HOS test results was normal (59 ± 9). However, when the sperm motility results was abnormal (32 ± 13.9) the HOS test results was lower (53 ± 0.12). In patients with normal sperm concentration (97.5 ± 6.7), the HOS test results was normal (66 ± 10), while when the sperm concentration was abnormal (7.27 ± 4.7) the HOS test results was lower (46 ± 0.11). In patients with normal sperm concentration but lower motility the HOS test results was abnormal (59 ± 9%). However, in patients with normal sperm motility but lower sperm concentration the HOS test results was normal (61 ± 0.04) not reaching significant differences. There were no correlation between the HOS test and sperm concentration and motility.

P1/2 - 068
ANXIETY AND CHANGES IN SPERMIOPGRAMM PARAMETERS
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INTRODUCTION: There are conflicting reports regarding the association between anxiety and sperm parameters. The purpose of this study is to determine the relationship between psychological stress and spermogram parameters MATERIAL AND METHOD: Sperm samples were obtained from 47 healthy young men (Trakya University Medical School second grade students) aged between 18-23 (mean:20±1.11). Prior to their anatomy final exam the students were evaluated by Spielbergier adults anxiety condition test. Spermogram (Volume, % motility, sperm count (density), % normal morphology) were performed simultaneously. Prior to and after the exam the difference between the variables was evaluated by the student-t test and the average anxiety scores. The relationship between anxiety scores and spermogram parameters was evaluated by Pearson correlation analysis (X2 test). RESULTS: The anxiety scores prior and after the exam calculated noted a significant difference (t=8.473, p<0.000). A statistically significant difference was found between average % sperm motility. The difference between average normal and abnor- mal % sperm morphology was also statistically significant. Our results demonstrated that there is an increase in % normal sperm morphology after the exam. CONCLUSION: The exam anxiety is an important stress factor for the students. Our results support previous studies reporting neg- ative effects of the physiological stress on spermogram parameters. Etiology and stress factor in the treatment of the infertility patients should be taken into consideration while adapting these results in the clinical practice.
P1/2 – 069
EVALUATION OF TISSUE FACTOR AND INTERLEUKIN-6 LEVEL IN SEMINAL PLASMA
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Introduction: The presence of tissue factor (TF) has been previously reported in human semen. Thus, TF in semen could play an important role in the defense of spermatozoa against cellular immune attack in the male seminal tract. Interleukin-6 (IL-6) is thought to promote inflammatory protein synthesis and it is a marker of the immune system activation. Further more, TF and IL-6 have been suggested to play an important role in the cross-talk between the cytokines and coagulation cascade. Materials and Methods: We measured the seminal plasma levels of TF and IL-6 in males by one-step sandwich ELISA using a commercial kit (The Chemo-Sero-Therapeutic Research Institute, Kunamoto-city, Japan and R & D systems, Minneapolis, MN, U.S.A.), and examined their relationship with sperm concentration and motility. This study comprised 71 patients categorized in three groups: infertile group with (n=11) and without (n=50) leukocytospermia, and fertile group (n=10). Results: Seminal plasma levels of TF were significantly higher in the infertile group than in the fertile group (p<0.05). Further more, seminal plasma levels of both TF and IL-6 were significantly higher in infertile patients with leukocytospermia than in those without leukocytospermia (both, p<0.05). Seminal plasma levels of TF were significantly correlated with the sperm (r=0.381, p<0.01). Conclusions: These findings suggest that increased TF levels in seminal plasma may be induced by inflammatory mediators and that this increased TF may be the cause of low sperm motility and infertility.

P1/2 – 070
SEMINAL MICROFLORA IN INFERTILE AND CHRONIC PROSTATITIS PATIENTS.
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Routine bacteriological methods cannot reveal the full complexity of seminal fluid microflora. Our aim was to prove the need for the quantitative full-microflora semen analysis for determining the role of infection in infertility and chronic prostatitis. We investigated the seminal microflora in 65 men: 40 of them had clinic picture of chronic prostatitis (Group I), and 25 appeared due to infertility of the couple. By cytological analysis, 13 men of the last group had (Group II) and 12 men did not have leukocytospermia (Group III). Semen samples were cultivated for detecting anaerobic (Wilkins-Chalgren and Schaeder media, Oxoid), aerobic (freshly prepared blood agar) and microaerophilic microorganisms (Gardnerella vaginalis selective agar, and MRS agar for lactobacilli, Oxoid). M. hominis and U. urealyticum were detected using Mycoplasma IST test (BioMerieux). N. gonorrhoeae and C. trachomatis were detected by PCR method using Amplicor C. trachomatis/N. gonorrhoeae Test (Roche). No sterile sample was found. The patients of the Groups I and II had 3...8 (median 5) different microorganisms in their semen, the number of microorganisms was 102...107 (median 105) per ml. The Group III appeared different: 1...7 (median 3) different microorganisms in the semen, the number of microorganisms 102...107 (median103) per ml. High frequency of anaerobic microorganisms was found (in 75% of the samples in Group I, 85% in Group II and 67% in Group III). The most frequently isolated microorganisms were staphylococci, corynebacteria, peptostreptococci, streptococci, anaerobic Gram-negative rods. U. urealyticum was found in high numbers more frequently in Groups I and II. No semen sample showed infection by N. gonorrhoeae or C. trachomatis.

P1/2 – 071
EFFECTS OF BACTERIA ON SEMEN PARAMETERS.
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According to WHO criteria, seminal fluid (SF) culture should be done to assess the presence of bacteria. This study was aimed at developing a simple method of assessing the presence of bacteria in fresh seminal fluid. Objectives: - a. To establish a reliable method other than seminal fluid culture and ABST to assess the bacteria in seminal fluid, b. To show the correlation between the presence of significant amount of bacteria (according to our new method) and the seminal fluid parameter abnormalities. c. To show the improvement of the seminal fluid parameter abnormalities, associated with the presence of significant amount of bacteria, after antibiotic treatment. Methodology: 294 males were referred to our Andrology laboratory during February 1998 and December 2000 were selected. There Seminal fluid analyses were done according to WHO lab manual (4th edition ). In Addition, the presence of bacteria in fresh semen samples were assessed by phase contrast microscopy, under power 20. We graded the presence of bacteria as follows - (+) given for occasional bacteria, (+++) given for colonies of bacteria and (++++) given for field full of bacteria per high power field. For our study, (+++) was taken as significant amount of bacteria. Patients who showed parameter abnormalities with significant amount of bacteria were treated with antibiotics and observed for improvement. Results:- The mean age group of the study population was 36 to 40 years. Out of 294, 195 (66%) had normal sperm concentration (20 million/ml or more). 43 showed mild oligozoospermia, 15 showed moderate oligozoospermia, 13 showed sever oligozoospermia and 28 were azoospermics. Out of 195, who had normal sperm concentration, 97 (50%) were normozoospermic, but 21 (21%) of them had significant amount of bacteria. 98 (50%) out of 195 males were not normozoospermic.

P1/2 – 072
FLOW CYTOMETRIC METHODS TO MEASURE HUMAN SPERM CONCENTRATION
The routine assessment of sperm concentration by haemocytometry (HM) is subject to relatively large random errors. We used three flow cytometric methods for human sperm counting from a 1:1 mixture of a diluted sample with a suspension of fluorospheres of known concentration. The events supposed to be sperm cells were detected according to: 1) gating on size and granularity (FCM1), 2) gating on DNA staining by propidium iodide (FCM2), and, 3) a combination of FCM1 and FCM2 (FCM3). The sperm concentration was calculated from the ratio of detected events to the fluorospheres count and concentration. A pilot study undertaken only for FCM1 which was compared to HM performed by 12 technicians from different laboratories showed a general agreement between both methods despite wide variations in sperm concentration by HM. A second experiment indicated that the overall variability in sperm concentration assessment by FCM1 was lower than by HM when performed by two technicians using optimal procedures for three preparations of the same semen samples. The overall mean coefficients of variation were 3.9% for FCM1 vs 8.0% for technician 1, 12.3% for technician 2 (p<0.05) and 15.7% for both (p<0.05). FCM1, FCM2 and FCM3 were compared to HM performed by a single trained technician for 39 semen samples (triplicates) of various quality. In comparison to HM, FCM1 and FCM2 overestimated the sperm concentration by 14% and 8% against only 4% per million sperm for FCM3 which was effective on the full spectrum of sperm concentration (except for azoospermia). In conclusion, this study shows that human sperm concentration can be accurately assessed by the flow cytometric method combining gating on cell size, granularity and DNA staining suggesting its potential for quality control and multicentre studies.
Abstracts – Poster Session 1/2

P1/2 – 073
CHARACTERIZATION FROM SPERM CAPACITATION IN Agouti paca AND Agouti taczanowskii.
A. paca live in America, in lowlands ranging from southern Mexico to northern Argentina and A. taczanowskii is found in the high cloudy forest of the Andean cordilleras of Venezuela, Colombia, and Ecuador. They are hystricognath rodents producing litters of 1 or 2 babies after a gestation of 115 days; the alimentary habits include fruit, nuts and seeds; they are important seed spreaders. Humans are the most important predator due the quality of the meat and this has led to their classification in “watch priority”. The main purpose of this project was to characterize the sperm, in order to use it in IVF programs. Concentration and motility was evaluated. After separating the motile fraction through a percoll gradient, heparin induced capacitated sperm was double stained with FITC-propidium iodine to determine membrane integrity, capacitation and acrosome reaction. Analysis in a completely random design and the Tukey’s test for significance of average values (p<0.05) were used. Semen concentration and motility for A. paca and A. taczanowskii was similar 4.8x108 esp/ml and 50-70% motile sperm. Posperecral evaluation showed 47.5% intact membranes, 25.7% capacitated and 28% spontaneous acrosome reacted A. paca sperm and similar results were found for A. taczanowskii, 48.5%, 32% and 20%, respectively. After heparin induced capacitation we found 22% intact sperms, 61% capacitated and 16% acrosome reacted in A. paca.

P1/2 – 074
SPERM CHROMATIN STRUCTURE ASSAY (SCSA) SHOWS CORRELATION WITH SPERM MORPHOMETRY
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Although male infertility is a common problem, predicting fertility is difficult. Routine semen analysis involves microscopic assessment that is inconsistent, poorly standardised and highly subjective. Therefore, it is important to devise objective methods of semen analysis, such as SCSA. Evenson et al have shown that SCSA correlates with fertility in animals and humans, independently of standard semen analysis. It uses flow-cytometry to assess the chromatin structure of abnormal sperm following treatment with an acid solution. Denaturation is measured using the dye parametric dye acridine orange, which fluoresces green when bound to normal DNA and red when bound to abnormal DNA. The overall aim of this study is to examine the relationship between SCSA, standard and computer-assisted semen analysis, and fertility rates in normal men, infertile men and with IVF. Preliminary results indicate that SCSA analysis is not affected by a delay in semen storage or by the presence of bacteria, except at very high concentrations. SCSA analysis is reproducible within (CV 11%, n=10) and between (CV 13%, n=10) samples for an individual. The relationship between SCSA and standard analysis was verified (n=200), showing significant correlation with vitality (r² 0.218), motility (r² 0.308) and morphology (r² 0.214). Interestingly SCSA is also significantly correlated with the automated sperm-head morphometric analysis parameters (r² 0.33 - 0.18) that are important for sperm-zona pellucida binding, which in turn is highly correlated with fertilization rates in-vitro.

P1/2 – 075
A NEW PROCEDURE FOR DETECTING DNA FRAGMENTATION IN HUMAN SPERMATOZOA USING THE TUNEL METHOD
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Introduction During spermatogenesis, some chromosomal damage is not restored after meiosis, and gene deletions are found in ejaculated sperm. In this study, we devised the procedure for detecting DNA fragmentation in damaged sperm using the TUNEL method. Materials and methods Semen specimens were collected by masturbation (n=129, 60 from infertile patients and 60 from volunteers). An aliquot of semen corresponded to 10 million sperm was trapped on a membrane filter (pore size: 3 micrometer) instead of conventional smear on a glass slide. We examined the DNA fragmentation by the TUNEL method both for ejaculated sperm and purified progressively motile sperm collected by a continuous-step density gradient in 89% Percoll and subsequent swim up method. Results When compared the rate of sperm with DNA fragmentations according to their seminal findings, it was significant lower in normozoospermia (9.4±/6.2%, n=73) than that in oligo-as�enozoosperasia (25.9±/16.0%, n=24). On the other hand, the rate in purified sperm (n=3) was decreased to 0.4±/0.5%. Conclusion The rate of sperm with DNA fragmentation was significantly higher in oligo-as�enozoosperasia than in normozoospermia and it was extremely low in purified motile sperm, which shows that the DNA fragmentation is valuable parameter for evaluating sperm quality. The new TUNEL method applying trapped sperm on a membrane filter is simple and useful procedure for detecting the fragmentation of sperm DNA. We also ascertained that sperm with very little DNA fragmentations useful for ICSI could be collected by sperm purification.

P1/2 – 076
LABORATORY METHODS FOR THE DIAGNOSIS OF ASTHENOSPERMIA
Asthenosperma, or low sperm motility, is a common cause of human male infertility. Semen values must be carefully interpreted since wide fluctuations of motility as well as limitations and inaccuracies of the various methods and the investigators bias the results. The aim of this study was to compare the conventional laboratory techniques with the computer-assisted system in assessing the diagnosis of this disease.
Human sperm samples from 46 men were used in this study. All samples were evaluated for standard semen parameters by two trained technicians, according to WHO criteria. A computer-aided semen analyzer (CASA, HTM-IVOS) was employed. The data on sperm motility were analyzed by Student-t-test: the McNemar-Chi-square test was carried out to ascertain the diagnostic results.
The overall mean coefficient of variation for the two participants and the 46 samples studied was 17.5% for the percentage of progressive motile spermatozoa (r=0.77). The mean progressive motility (%) was 40 ± 10 and 41 ± 23 by conventional and CASA analysis respectively (p: NS), while data on grade a spermatozoa showed significant difference between the methods employed (15 ± 10 and 31 ± 21, p<0.000001). Twenty-nine (63%) samples were classified as asthenospermic by both methods. From the studied samples, 12 (26%) were classified as asthenospermic by the subjective method and 2 (4.3%) by CASA (p=0.01).
The present study provides evidence that the two methods do not provide directly comparable data.
P1/2 - 077

EFFECT OF THE SEMEN COLLECTION PLACE ON THE SEMEN QUALITY
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To evaluate the effect of the semen collection place on the semen quality, we compared semen parameters collected in the lavatory and those collected in the private room. Semen samples were collected from each ten healthy volunteer via masturbation in the lavatory and additional semen samples were in the private room. The semen analyses were performed as recommended in the WHO manual (1992). The parameters assessed were semen volume, sperm concentration, progressive sperm motility, the proportion of morphologically normal sperm and the percentage of viable sperm. The difference in the value of sperm concentration between samples collected in the lavatory or in the private room was not significant (86.8±25.4×10^6/ml (mean±SD, median: 83.9×10^6/ml) and 97.1±72.0×10^6/ml (mean±SD, median:76.0×10^6/ml) respectively). The values of the other semen parameters were also not significant between the places. These results suggest that the semen collection place does not affect the semen quality. Granted by Environmental Agency of Japan and Japan Society for the Promotion of Science.

P1/2 - 079

REPRODUCTIVE STATUS OF MALE GIANT PANDAS
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There are ~120 giant pandas living ex situ in China and probably ~1,200 pandas living in fragmented populations in nature. As a first step to developing a self-sustaining ex situ population, a Biomedical Survey of giant pandas was conducted in China in February and March (breeding season) 1998, 1999 and 2000 to assess the health, reproduction, behavior, genetics and nutrition of 61 individuals (24 males, 37 females). Sixteen males (5 to 16.5 y) were anesthetized for reproductive assessments. Data on reproductive history revealed that 11 of the 16 (69%) males had never mated naturally. All males produced sperm ejaculates (mean, 1.2±10^6 sperm/ml, 69% sperm motility, 66% normal sperm; 90% intact acrosomes) Spem morphology was influenced by age. There were fewer (P<0.05) normal sperm in the four 5.5 y old males (31%) compared to the 12 males that were 6.5-16.5 y (77%). Two males had a unilateral inguinal testis of smaller size (testis volume, 24 and 76 cm^3) than the descended normal testis (210 and 152 cm^3, respectively). Semen quality in these two males was comparable to males with two normal testes. There was no difference (P>0.05) in ejaculate traits in wild-born (n = 7) versus captive-born (n = 9) pandas or between breeding (n = 9) and non-breeding (n = 11) males. These data reveal that (1) giant pandas held ex situ generally produce prodigious amounts of motile, structurally normal spermatozoa during the breeding season, (2) males as young as 5 yr produce sperm and (3) there is a hypoplastic testicular condition of unknown etiology in a subpopulation.

P1/2 - 080

INFLUENCE OF H+ CONCENTRATION ON THE VIABILITY OF A23187-TREATED HUMAN SPERMATOZOA
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Introduction: The calcium ionophore A23187 is used to induce the acrosome reaction of spermatozoa. Reduction of the motility of spermatozoa by A23187 had been investigated. We examined the influence of pH on the viability of human spermatozoa treated with A23187.

Materials and Methods: 3 semen specimens with normal parameters according to WHO-standards were pooled, washed and resuspended with Ham's media adjusted to pH 6.0, 6.5, 7.0, 7.5, and 8.0. The samples were divided and incubated with A23187 or solvent for 1h in sealed vials as well as in CO2-atmosphere. Dead spermatozoa were stained with propidium iodide and detected by flow cytometry. The acrosomal status was assessed by lectin staining with pisum satium agglutinin.

Results: At different pH 30% of spermatozoa, treated with solvent only, were vital. The rate of avital spermatozoa in samples treated with A23187 in air exclusion increased with increasing pH from 38% to 97%, while the increase was reduced in CO2-atmosphere from 30% to 65%. As some of the avital spermatozoa loose their acrosome the rate of acrosome-absent spermatozoa increased with higher pH. Conclusions: With increasing pH A23187 showed more toxic effects to human sperm. The transmembrane pH-gradient is important for the exchange of Ca^2+ against H^+ by the ionophore. Accumulation of the anionic form of A23187 may be responsible for the toxic effects. A23187 is not a physiological inducer of the acrosome reaction like zona pellucida protein but readily available.
**P1/2 - 081**

FUNCTIONAL TESTS AS THE MARKERS OF FERTILIZING CAPACITY OF SPERMATOZOA
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In order to provide complete information on fertilizing capacity of spermatozoa numerous functional tests have been used. The functional tests include study of the function of spermatozoa, their membranes, enzyme potential, morphological forms and acrosome reaction. The aim of the study was to investigate fertilizing ability of sperm of our 100 infertile and 20 fertile men by determination of spermogram parameters: the sperm count, motility, morphology, vitality and HOS test according to procedure recommended by WHO, acrosome reaction was studied with monoclonal antibodies GB24 and acrosomal staining (modified Papanicolaou method) was used for evaluation of the size and shape of acrosomal cap of spermatozoa. The results were subjected to statistical analysis by multifactorial discrimination analysis. Discriminatory analysis with the adopted criterion that Z<0 are fertile and Z>0 infertile men suggest good fertilizing ability of patients with patients whose spermogram showed >0.70 normal morphological forms, >0.34 rapid 'a' spermatozoa, <8% spontaneous acrosome reaction (SAR), <0.12 pathological spermatozoon heads, <0.09 pathological tails, >0.50 progressively mobile spermatozoa (a+b), >0.75 living spermatozoa and >26% induced acrosome reaction (IAR). These results have confirmed the complexity of spermatozoon function that can be defined only by the simultaneous use of multiple parameters.

**P1/2 - 082**

THE HUMAN SPERM HEAD, A KEY FOR SUCCESSFUL FERTILISATION
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Introduction: It has been recommended that in the morphological assessment of human sperm the most important region to study is the sperm head. Evaluation of the sperm head is multiparametric and entails a study of the sperm head shape and size, the acrosomal size, vacuoles as well as testing the integrity of the acrosome reaction. Aim: To examine the predictive value of determining the sperm head shape, acrosomal size, the presence of acrosomal vacuoles and the challenged acrosome reaction on the outcome of a standard IVF programme. Materials and methods: Included in this study were 95 couples. After routine semen analysis two fresh slides were prepared for each patient and stained with the Spermac, stain. Assessment of sperm morphology including sperm head shape and size and detailed acrosomal morphology (acrosomal size and vacuoles) was performed using the Hobson Sperm Tracker, Sheffield, UK. The acrosome reaction was detected using FITC-labelled Lectin, Pium Sativum Agglutinin (PSA). The assessment of the acrosome reaction was done before and after adding pooled undiluted human follicular fluid (FF). Results: A positive correlation was found between the fertilisation rate, FR%, and the proportion of the sperm exhibiting: normal (oval) head shape (P<0.001), acrosomal vacuoles (P<0.003), normal acrosomal size (40-70%) (P<0.025) and the percentage of acrosome reaction after adding FF (P<0.001). Multiple logistic regression analysis revealed that by incorporating the above four parameters, the sensitivity of prediction of the IVF success is 79%, the specificity is 93% and the positive predictive value is 96%. Conclusion: This study shows that the multiparametric assessment of sperm head is useful in predicting the success rate of a standard IVF.

**P1/2 - 083**

THE EFFECT OF SPERM WITH HYPO-Osmotic SWELLING TEST (HOST) SCORES IN THE GREY ZONE AND SUBSEQUENT TREATMENT WITH CHYMOTRYPSIN ON IN VITRO FERTILIZATION (IVF) OUTCOME.


There is marked subfertility noted in women whose male partners have HOST scores <50%. The defect caused by a subnormal HOST test is not fertilization failure, but poor implantation. One study using one common pool of shared oocytes for donor recipients found a clinical pregnancy rate (PR) following IVF-ET of 50% when the HOST score was ≥50% and "0%" when <50% despite transferring the same number of morphologically similar embryos. Previous in vivo studies did not find any subfertility associated with the grey zone of 50-59%, but possibly with the zona pellucida exposed to much higher concentrations of sperm with IVF; sperm with HOST scores in the grey zone may be associated with decreased fecundity. Furthermore, since some pregnancies in vivo have been achieved by IUI by first treating sperm with low HOST scores with chymotrypsin, the study would also evaluate whether such treatment would improve PRs. Patients were offered the option of chymotrypsin treatment of sperm or standard preparation. The viable PRs were 44.4% (4/9) in those electing chymotrypsin treatment vs 50.0% in controls. Implantation rates were 20.0% and 20.7%, respectively. These data do not show that sperm with HOST scores in the grey zone are associated with decreased fecundity following IVF-ET and chymotrypsin does not help or hurt.

**P1/2 - 084**

THE RELATIVE DISCREPANCY BETWEEN VIABILITY AND HYPO-Osmotic SWELLING TEST (HOST) SCORES IS NOT RELATED TO IN VITRO FERTILIZATION (IVF) OUTCOME.


Sperm with subnormal HOST scores contains a toxic factor that impairs the functional integrity of the sperm membrane. This defect does not impair fertilization, but inhibits implantation. Clinical studies find only rare pregnancies from males with HOST scores <50%, but normal above this level. A low HOST score does not necessarily indicate poor viability which measures the structural integrity of the sperm membrane, but if the membrane is structurally damaged, then functional impairment is likely. The present study evaluated the possibility that some sperm may have the toxic factor present but with high viability levels, the HOST score may not drop below the 50% level. IVF-ET cycles were thus evaluated according to the difference between viability and HOST score. A relative discrepancy score was computed as relative discrepancy = (viability-HOST score)/viability. The discrepancy scores ranged from 2.3 to 40.4. The 25th percentile was 9.1, 50th percentile 16.1, 75th percentile was 23.9. The patients were stratified into 4 groups based on the 4 quartiles of the discrepancy score. The clinical and viable pregnancy rates (PRs) did not differ within the 4 quartiles (from least to most) (46.7, 28.6, 50.0, 40.0% for clinical PR and 40.0%, 21.4, 35.7, 33.3% for viable PR). Implantation rates were also smaller (21.7, 15.5, 21.4, 17.9%). These data do not support the hypothesis that the relative discrepancy between HOST score and viability is related to IVF outcome.
P1/2 - 085

SPERM NUCLEAR DECONDENSATION ASSESSED BY CONFOCAL MICROSCOPY AND FLOW CYTOMETRY IN HUMAN INFERTILITY.

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When a spermatozoon enters an egg, sperm chromatin decondensation occurs. Defective chromatin decondensation may prevent development of male pronucleus. Aim of study: To present procedures for detecting sperm samples unable to decondense. Material & Methods: Pooled normal control semen and samples from unexplained, idiopathic, varicocele and paraplegic patients were tested. Human sperm decon-densation was observed under fluorescent confocal microscopy in propidium-iodide stained hamster eggs after hamster test (SPA) or microinjection (ICSI). Sperm decondensation was also investigated using the Montag in vitro decondensation procedure and acidine-orange flow cytometry scatter-grams done during the procedure. Results: In SPA, decondensed sperm heads were found peripherally. In failed SPA case, confocal microscopy showed penetrated sperm heads unable to decondense. In hamster microinjected eggs with human sperm, <10% of sperm heads decondensed. In normal sperm, flow cytometry scattergrams done within the 30 min period of in vitro decondensation showed a gradual increase of green and red fluoro-cence with a gradual increase of decondensed cells. In most samples decondensation was normal. In 3/6 paraplegic patients and 1/3 with failed hamster test decondensation failure was assessed. Conclusion: Nuclear decondensation assessment using confocal microscopy and flow cytometry help better understanding the significance of failed SPA or ICSI. These methods appear valuable and complementary in assessing sperm chromatin decondensation and should be considered useful additions to the severe male infertility work up, especially where ICSI is mandatory.

P1/2 - 087

COMPARISON OF SEMEN CYTOLOGY & DIAGNOSTIC TESTS ON BIPSY IN DIAGNOSIS OF MALE INFERTILITY

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Objectives: To evaluate the correlation between simultaneous semen cytology and diagnostic testis biopsy in diagnosis of male infertility.

Design: Azospermia patients (n=122) and volunteers (fertile males) (n=50) were recruited in this study for simultaneous diagnostic testis biopsy and semen cytology. Setting: Samples were collected from subjects referred to Nanjing Jinling Hospital infertility clinic. Patients, participants: One hundred seventy two semen samples and testicular specimen from 172 patients and healthy men were analyzed. Main Outcome Measures: Sperm production is a measurement of testicular spermatogenesis and the obstruction of seminiferous tubules, therefore, a positive correlation between semen cytological analyses and diagnostic testis biopsy was anticipated. Results: Azospermia patients showed 91% correlation between semen cytological results and testis biopsy results. Results obtained from two diagnostic methods showed significant correlation. In 14 of 122 patients, sperm and spermatogenic cells appear in testis biopsy while no spermatogenic cells appeared in semen cytological analyses. Further biochemical data of semen analyses demonstrated that 13 cases were due to the obstruction of seminiferous tubules. One was due to reverse ejaculation. Conclusions: Sperm production measured by diagnostic testis biopsy and semen cytological analyses showed fair consistency, suggesting that semen cytology could be an ideal attraumatic alternative method to replace the testis biopsy method to assess the sperm production of testis and the obstruction of seminiferous tubules in diagnosis of male infertility.

P1/2 - 086

ROLE OF THE SPERM CHROMATIN STRUCTURE ASSAY (SCSA) IN THE HUMAN INFERTILITY CLINIC

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Approximately half of infertile men are classified with idiopathic infertility. Some unknown percentage of of these idiopathic cases are due to damaged sperm DNA. Over the past decade, various tests such as the TUNEL, COMET and CMA3 assays have been used to identify sperm chromatin abnormalities that relate to infertility and/or unsuccessful pregnancies. These tests, however, are mostly light microscopic tests that provide marginal statistical power, are slow and subject to significant intra- and inter-observer variations. In sharp contrast, the flow cytometry-based SCSA measures in seconds 5,000 individual sperm that have been subjected to low pH to potentially induce DNA denaturation that is detected by acidine orange staining (COMP population). These data are statistically robust, totally unbiased and have higher repeatability (98-99) than any commonly used sperm quality assay. Rapidly accumulating data show that the SCSA is strongly predictive of semen samples not being compatible with in vivo or in vitro derived fertility and/or sustained pregnancy. Previous human fertility SCSA studies set a threshold of 30% as the upper limit of sperm with damaged DNA (COMP); no successful pregnancies were observed by IVF, ICSI or in vivo attempts. More recent data have suggested a lower threshold of about 25% COMP. Pregnancies that have occurred with COMP above that threshold have resulted in spontaneous abortions. Our current data strongly suggest that an infertility clinic would better serve their patients if the SCSA was offered to determine if the sperm DNA integrity is sufficient to support successful pregnancy. Supported by EPA R827019.

P1/2 - 088

SPERM CHROMATIN STRUCTURE ASSAY DOES NOT PREDICT EARLY EMBRYO DEVELOPMENT IN VITRO

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Introduction. There is growing evidence that the integrity of sperm chromatin may be predictive for male fertility potential. In particular, Sperm Chromatin Structure Assay (SCSA) increased COMP values (the fraction of cells with abnormal chromatin) are considered not compatible with good fertility in vivo. However, there is little known at which stage of embryo development, especially with assisted fertilization in vitro, defective sperm chromatin structure might have an adverse effect. We present preliminary observations on relation between SCSA and blastocyst formation in vitro.

Materials and Methods. Two consecutive semen samples (pre-treatment evaluation and treatment sample) from males of 71 couples attending infertility clinic were evaluated according to WHO99 guidelines. Additionally, on frozen semen aliquots SCSA was performed according to the original protocol. In total 383 fertilized oocytes (standard IVF and ICSI) were followed up to the fifth day (D5) when blastocyst formation was recorded as expected outcome. Binary logistic regression analysis was performed to evaluate the relation between all measured variables and blastocyst formation on D5.

Results & Conclusions. SCSA COMP value in either semen sample is not predictive for fertilized oocytes ability to form a blastocyst on Day 5 of culture in vitro (logistic regression P = 0.366 and 0.4211 respectively). Although male genome is though to contribute to embryo development from D3, it seems the damage to sperm DNA measured by SCSA does not adversely influence embryo development at this stage.

Abstracts – Poster Session 1/2

P1/2 – 089
ULTRASTRUCTURAL ANALYSIS OF CHROMATIN DEFECTS IN TESTICULAR SPERMATIDS OF AZOOSPERMIC MEN SUBMITTED TO TESE-ICSI
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TESE-ICSI is offered to treat obstructive and non-obstructive azoospermia, although factors that influence the outcome of ICSI are not well defined. It was determined, at ultrastructural level, the percentage of elongated spermatids with a normal condensation of chromatin in azoospermic patients submitted to TESE-ICSI. The ultrastructural data were compared to parameters of efficiency of spermatogenesis including the number of elongating spermatids, the number of degenerating germ cells per 100 Sertoli cell nuclei, and the serum level of FSH. The quantitative analysis could be applied to 9 biopsies classified as post-meiotic arrest of spermatogenesis (PMA), and compared to 10 biopsies with apparently normal spermatogenesis. The percentage of elongated spermatids with normal chromatin in PMA was lower than that observed in cases with normal histology (mean: 4.4, range: 0-20, and mean: 52.9, range: 40-70, respectively). The percentage of elongated spermatids with normal chromatin condensation was negatively correlated with the serum level of FSH (R=-0.86, p<0.001) and the number of degenerating germ cells per 100 Sertoli cells nuclei (R=-0.68, p=0.001), while it was positively correlated with the number of elongating spermatids per 100 Sertoli cell nuclei (R=0.81; p=0.001). The percentage of elongated spermatids with normal chromatin condensation was not correlated with the rate of oocyte fertilization (R=0.08), while the delivery rate/cycle was higher in cases with normal histology compared to cases of PMA. An altered chromatin condensation is a ubiquitous defect in spermatids of non-obstructive, azoospermic men submitted to TESE-ICSI.

P1/2 – 090
MOLECULAR CONTROL OF FERTILIZING POTENTIAL OF MALE GAMETE IN AZOOSPERMIA: A STUDY OF GENES ENCODING RBM, DAZ, AND TSPY.

Three non-overlapping regions of microdeletions in Yq11 (AZFa, AZFb (RBM), and AZFc (DAZ)) have been identified that are probably responsible for azoospermia or oligozoospermia. The Y-chromosome microdeletions cannot be predicted on the basis of clinical findings or the results of semen analysis. Another gene family TSPY (Testis-Specific protein, Y-encoded) is located on the short arm of the Y chromosome at Yp11.2. This gene is expressed in spermatogonia and primary spermatocytes in adult males. The aim: to study the genes encoding RBM, DAZ and TSPY in functional azoospermic males. Method: RNA was extracted from testicular cells using QuickPrep total RNA extraction kit (Pharmacia) for 15 azoospermic patients. DNA was extracted from blood using GenomicPrep DNA isolation kit (Pharmacia) for 50 azoospermic patients. PCR was done on the DNA and RT-PCR was done on the testicular RNA using specific primers. To amplify the DAZ gene, BestFit alignment between the DAZ on the Y chromosome and DAZLA on chromosome 3 was done. Results: In Egyptian functional azoospermic males there is high frequency of deletion of DAZ, RBM and TSPY genes (28%, 20% and 4% respectively). The methodology to detect these mutations using gene specific primers was successfully established. Gene expression in testicular tissue of azoospermic males was also evaluated and failure to detect the message correlates with the phenotype.

P1/2 – 091
SERTOLI CELL FUNCTION IN INFERTILE PATIENTS WITH AND WITHOUT Y CHROMOSOME MICRODELETIONS
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Introduction. Deletions on the Y chromosome long arm (Yq) are a frequent cause of male infertility. No clinical or hormonal parameter have yet been found to distinguish patients with and without Yq microdeletions. In particular Sertoli cell function, as evaluated by inhibin B concentrations, has not yet been described. We evaluated the testicular hormonal function in patients with and without Yq microdeletions. Materials and methods. We studied 102 severely infertile patients, selected by means of seminal analysis, FSH and inhibin B concentrations, testicular FNAC. Yq microdeletions analysis by PCR using 40 STSs and primers specific for DAZ, RBM, USP9Y and DBY genes. 27 patients had Yq microdeletions and 75 were idiopathic. Results and discussion. Patients with Yq microdeletions had lower FSH and higher inhibin B plasma concentrations than patients without microdeletions, suggesting that Sertoli cell function in Yq deleted men is only partially altered. Patients with deletions of germ cell-specific genes (DAZ, RBM) had higher concentrations of inhibin B than patients with deletions of ubiquitously expressed genes (USP9Y, DBY), suggesting that when a specific alteration of germ cells exists Sertoli cell function is only partially damaged. In patients without deletions the cause that has determined the spermatogenic defect may have damaged both Sertoli and germ cells. This study elucidated the multifactorial mechanisms underlying spermatogenic defects, where Sertoli cells may be normally functioning or altered depending on the primary cause that has determined the testicular damage.

P1/2 – 092
SCREENING FOR THE PRESENCE OF THE AZF (AZOOSPERMIA FACTOR) CANDIDATE GENES IN IDIOPATHIC AZOOSPERMIA AND OLIGOZOOSPERMIA
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Introduction and objectives. Approximately 10% of males with idiopathic azoospermia or oligozoospermia have microdeletions in Y chromosome factors (AZF) region. Three distinct regions, designated AZFa, b and c from Yq are required for spermatogenesis. In each region, candidate spermatogenic genes have been proposed. The aim of this study was to screen azoospermic and oligozoospermic patients for deletions of known specific Y chromosome genes, namely DFRY (Drosophila fat facets related Y), DBY (DEAD/H box polypeptide, Y chromosome), UTY (ubiquitously TPR motif Y), CDY (chromodomain Y), XRY (XK related Y), SMCY (selected mouse cDNA on the Y), eIF-1AY (translation initiation factor 1A), RBM (RNA binding motif), DAZ (deleted in azoosperma), PRY (PIT-1 related Y), BPY2 (basic protein Y2). Methods. The patients consisted of 60 azoospermic, 40 severe oligozoospermia (<5x10^6 spermatozoa/ml). Genomic DNA was obtained from peripheral blood leukocytes. Each man was analyzed for the presence of 13 gene specific oligomers spanning the three AZF regions: DFRY, DBY and UTY (AZFa), CDY, XRY, SMCY, eIF-1AY and RBM (AZFb), DAZ, BPY2, PRY, BPY2 and CDY (AZFc). All patients were also performed the routine screening for about sixty STS (sequence tagged site) markers. Results: The loss of Yq specific genes was observed in 6 out of 100 samples (6%). The loss of genes was shown in 3% in eIF-1AY (AZFb), 2% in SMCY (AZFb), 3% in RBM (AZFb), 3% in DAZ (AZFc), respectively. These deletions were accompanied with the large deletion in the each AZF regions. Conclusion. This result suggested that gene-specific deletions using genome are rare events and only large Y deletion in the each AZF region, removing several genes, are associated with male infertility.
Abstracts – Poster Session 1/2

P1/2 – 093

FAMILIAL AZOOSPERMIA AND OLIGOSPERMIA
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Aim: The aim of this study was to evaluate the characteristics of related patients with oligo, or azoospermia. Patients from two institutions were evaluated and brothers and relatives were detected. A retrospective and prospective study was conducted.

Materials and Methods: Three hundred seventy eight patients were evaluated. The patients' history included familial occurrence of male infertility among brothers and uncles. Thirty five brothers and 3 uncles, were studied. Seven brothers and 6 uncles with a history of infertility could not be examined. The patients were evaluated by repeated semen analysis, FSH/LH/PRL/L levels, ultrasound, testicular biopsy and chromosomal analysis. Several brothers and uncles had Y deletion testing. Results: Among 378 patients with oligo and azoospermia we found 35 brothers suffering of male infertility who had 9 uncles treated for this problem. Seven additional brothers refused our study and 6 uncles could not be traced. Brothers of nine families had a similar semen analysis of oligo or azoospermia. Others had a mixed picture. The histology of the testicular biopsies revealed different types, except for two families with azoospermia. Three uncles that could be studied, had azoospermia and their genetic tests revealed an intact karyotype. Studies of Y chromosome deletions among 4 families showed 2 brothers with an AZFc deletion. Conclusions: Oligo, or azoospermia can be detected among brothers and uncles. Additional studies are needed to clarify the mechanisms.

P1/2 – 094

Y CHROMOSOME HAPLOGROUPS AND MALE REPRODUCTIVE FUNCTION
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Nordic-Baltic area is a fascinating region for investigations of male reproductive function. In this geographically close region, there are over 5-fold differences in the incidence of testis cancer, with highest incidence in Denmark and lowest in Baltic countries and Finland. Recent, yet unpublished studies have indicated that sperm concentration follows inversely the risk of testis cancer in this region. Population genetic investigations on Y chromosome diversity show major genetic barriers running along the Baltic Sea, indicating that the region was colonized by different popu-lations. Preliminary data from other populations show that male lineages with different structure of Y chromosome may vary in their sperm production and also probably in their ability to resist environmental factors that suppress male fertility. The Baltic countries and Finland differ from the rest of countries in Nordic-Baltic region by their high frequency (32-61%) of haplogroup (HG) 16. In our study we: 1) compared frequencies of Y chromosome haplogroups in general Estonian population (unknown fertility) and in men with severe semen pathology (= 5 mill/ml), and 2) investigated the influence of Y chromosome HG on incidence of certain genital pathologies. Comparing of two most frequent haplogroups, HG16 and HG3 in our population we found that in HG16 group there are less cases of unexplained semen pathology, higher testis volume and lower FSH values. The results of our study support the hypothesis that different structure of Y chromosome may influence male reproductive function. This emphasizes the importance of further studies on this topic in other populations and with larger number of haplogroups.

P1/2 – 095

CFTR GENE MUTATIONS IN INFERTILE MEN
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Aims: Cystic Fibrosis(CF), the most popular autosomal recessive genetic disorder in Caucasians (1/1600 descent), with carrier frequency 1/25, is caused by Cystic Fibrosis Transmembrane Conductance Regulator(CFTR) gene mutations(g.m.). Moreover,both alleles of CFTR gene mutations could determine Congenital Bilateral Absence of Vas Deferens(CBAVD). The aims of this work is evaluations of CFTR g.m. carrier state in different forms of male infertility. Materials & Methods: This report analyze 321 men,with no clinical signs of CFfrom infertile couples treated in Novum Center,before ICSI procedure:164-azoospermia,8-aspermia,103-cysto-azoospermia and 46-oligozoospermia(IFDS). Moreover,43 women from CFTR(-) husbands were tested. 10 CFTR gene mutations (dF508,G542X,N1301K,1717(+), W1282X,G551D,R553X,I507,R117H,IVS8-ST) were analyzed using InnoLipa tests and PCR technique. In 22/29 azoospermic men with CFTR gene mutation, testicular biopsies (percu-aneous, needle 1.6 G) were performed. Results: CFTR gene mutations were totally detected in 44 men(13.7%): azoospermia-29(16.7%), aspermia 1/(8.12.5%), cystoazoospermia 9/103(8.6%), OAT 5/46(10.9%) and in 5 women(11.4%). The most common CFTR g.m. was IVS8-ST(77.6%) and in 5 couples both partners had this mutation.In group with azoospermia and carrier state the positive (sperm cell were found) biopsies were reported in 16/22(72.7%) men. Conclusions: 1.In group with CFTR gene mutations and positive biopsy the cause of azoospermia is probably CBAVD, mild form of CF. 2.In all group of infertile men the carrier state of CFTR gene mutations is higher than normal men's population.

P1/2 – 096

ASSOCIATION BETWEEN THE HLA-DR AND DQ ALLELES AND MALE INFERTILITY
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Previous studies suggested that certain HLA alleles might predispose to non-immunological male infertility by conferring genetic susceptibility to disorders of spermatogenesis and/or male genital tract infections. We investigated the frequency of the HLA-DRB1 and HLA-DQB1 alleles in male partners of infertile couples since these alleles have been implicated also in autoimmunity. HLA typing was undertaken in 58 patients. DNA was extracted from semen after routine analysis and subjected to the polymerase chain reaction using sequence-specific primers (PCR-SSP). The frequencies of the HLA-DRB1*04, 010, 011, 015, 0301 and HLA-DQB1*03 alleles were found to be significantly higher in oligozoospermic patients than in the control population. In contrast, the HLA-DRB1*01301 allele was significantly lower in oligozoospermic patients than in controls. However there was no general association between any of the HLA-DRB1 or DQB1 alleles and the overall sperm density. It is concluded that HLA-DRB1*04, 010, 011, 015, 0301 and DQB1*03 alleles may be associated with male infertility and could play a role in disorders of spermatogenesis. We hope to confirm these findings in a much larger study.
P1/2 - 097
UTILITY OF INVESTIGATION OF ALLELES OF CFTR GENE IN INFERTILE MEN.

Cystic Fibrosis (CF), a recessive genetically inherited disease, can manifest itself as an isolated congenital bilateral absence of the vas deferens (CBVD) or as other urogenital malformations. The CF gene is named Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and the mutation most often observed is DF508. Over 95% of men with CF are infertile because of azoospermia. The objective of this study was to evaluate the frequency of mutations in the CFTR gene in infertile men with severe oligozoospermia or azoospermia (on at least two semen analyses), urogenital malformations or family history of CF. The concentration in blood of FSH, LH, PRL and Testosterone was determined in 38 patients. Abdominal and transrectal ultrasonography was performed. The Innolipa set was used for the diagnosis of alleles of CFTR gene. Each sample was screened for the most common mutations in the CFTR gene, based on the reverse hybridisation of amplified gene fragments. This set can identify over 70% of mutations in CFTR gene. In six of the 38 patients (15.78%) mutation DF508 was detected, connected to urogenital malformations in 5 cases (including 3 cases of CBVD). No other mutations were found. Because of the high frequency of mutations in the CFTR gene in these patients and the consequences in the treatment of infertility and genetic advice, we recommend this test.

P1/2 - 098
IS SCREENING FOR Y CHROMOSOME MICRODELETION IN PATIENTS UNDERGOING INTRACYTOPLASMIC SPERM INJECTION ALWAYS NECESSARY?
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Screening for Y chromosome microdeletion is recommended for all patients undergoing ICSI program although numerous data have shown that microdeletion frequency may vary from 0.4% to 55% depending on clinical criteria and methodology used. We reported herein a retrospective study from 1997 till 2000 of Y microdeletion screening on 408 infertile men with normal karyotype according to defined biological criteria: obstructive (n=17) and non obstructive (n=34) azoospermia related to FSH level and epididymal markers (carnitine and alpha 1-4 glucosidase), severe oligozoospermia (n=67; sperm count <1.10^9/ml), moderate oligozoospermia (n=68; sperm count 1-5. 10^9 /ml), mild oligozoospermia (n=41; sperm count 5-10^9/ml), severe teratozoospermia (typical spermatozoas < 10% according to David et al, 1975) and/or severe asthenozoospermia (motility < 10% n=143). A set of 17 Sequence Tagged Sites (STS) spanning the euchromatic region of Yq was tested for each patient to explore AZFa, AZFb and AZFc regions. Four of the 34 non obstructive azoospermic men (11.7%), 7 of the 87 severe oligozoospermic men (8%) and only 1 of the 86 moderated oligozoospermic men (1.2%) showed an Y chromosome deletion. None of the other infertile men had Y deletions. All patients had AZFc deletion and in two cases the AZFb region was also deleted. The Y microdeletions were observed only when sperm concentration was lower than 5.10^9/ml. Therefore the screening for Y microdeletion before ICSI should be restricted to selected infertile patients after andrological examination in case of non obstructive azoospermia or severe oligozoospermia.

P1/2 - 099

Some men with non-obstructive azoospermia (azoospermia/mild hydrospermia) have a Y chromosome micro-deletion and may have a lack of sperm available for ICSI. Although spermatozoa may not be present, it may be possible to obtain sperm from the testicles (testis biopsy or testis tissue biopsy) to obtain sperm with adequate motility and morphology suitable for ICSI. However, there is some debate concerning these cases. Some yield no sperm regardless of procedure. Furthermore, pregnancy data with acquired sperm from difficult cases is limited. To avoid unnecessary exploration, post-mortem analysis of exons 7 and 9 in microdissected DNA from testicular tissue (TESE) and spermatozoa is recommended. In this study, we have examined the frequency of microdeletions in infertile men, men with true azoospermia, and men with severe oligozoospermia (less than 1% of sperm motile). We report the frequency of microdeletions in infertile men and the genotyping of spermatogenesis genes in men with severe oligozoospermia. The results confirm the presence of microdeletions in men with severe oligozoospermia and the absence of azoospermia.

P1/2 - 100
INFLUENCE OF STRESS ON SPERM PARAMETERS OF INFERTILE MEN
S.Micic, N.Lalic*, N.Bojanic*, O.Durutovic*, I.Vukovic*, Clinic of Urology, Belgrade, Yugoslavia

Day to day life changes significantly of infertile couples after medical treatment begins. Their attention becomes focused on this singular failure, and other goals and needs are neglected. The aim of our study is to evaluate the sperm parameters in infertile men before, during and after the NATO intervention in spring 1999. There were 160 pts in the first group, those before, -160 pts during, second group and the third group consists of 135 pts after the intervention. We evaluate number, motility and morphology of the spermatozoa according the WHO manual. We divided all patients, to those with severe oligospermia oligospermia (5,1 to 20x10^6/ml) and normospermic (over 20,1x10^6/ml). Correlation between the groups in the different time was significant only in the group of severe oligospermic men. In this group of infertile pts we found significant decrease in number and in the motility of the spermatozoa, and also rising percent of abnormal spermatozoa. The number of spermatozoa from 2,4x10^6/ml decrease to 1,5x10^6/ml and percent of motile spermatozoas decrease from 8 to 4 (p < 0.05). The percent of abnormal spermatozoas raised from 55,6 to 65,6 (p < 0.05). In the other groups of infertile men we detected decrease in sperm parameters, but this difference was not significant (Fridman's test). In conclusion, we can say that the huge stress influence the most the group of severe infertile men.
**P1/2 - 101**

**THE AGING EFFECT OF MEN ON SEXUAL BEHAVIOR AND SEMINAL PROFILES: FACT OR FICTION?**


Objective: It has been shown that aging in women reduces the potential to produce quality oocytes and achieve conception. However, very little data show similar trends in men, possibly because spermatogenesis can continue throughout life. The aim of this study was to assess the seminal characteristics as well as the sexual behavior of men of various ages and to establish such an aging effect on those characteristics.

Materials and Methods: Semen samples were collected from men (*N* = 792) undergoing IVF or IUI in cases of female factor infertility only. Samples were collected via the use of a seminal collection device at intercourse and evaluated manually according to WHO standards (WHO). Men were divided into 4 groups according to ages: (i) 20-30, (ii) 31-40, (iii) 41-50, and (iv) 51-60, and their seminal characteristics and responses to a sexual behavior questionnaire were compared.

Results: The data showed statistically significant differences in all the seminal parameters tested, as well as their sexual behavior patterns in terms of the number of years they have been trying to achieve pregnancy and sexual satisfaction.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Volume (ml)</th>
<th>Concentration (mll/ml)</th>
<th>Motility %</th>
<th>Morphology</th>
<th>Years to Sex</th>
<th>Sex. Freq. (times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>33.0 ± 0.5</td>
<td>86.0 ± 3.6</td>
<td>69.0 ± 4.2</td>
<td>107.2 ± 2.7</td>
<td>31-40</td>
<td>63.0 ± 3.4</td>
</tr>
<tr>
<td>31-40</td>
<td>35.3 ± 4.0</td>
<td>72.5 ± 3.2</td>
<td>61.6 ± 4.4</td>
<td>101.1 ± 2.6</td>
<td>41-50</td>
<td>31.1 ± 2.6</td>
</tr>
<tr>
<td>41-50</td>
<td>31.3 ± 0.6</td>
<td>61.5 ± 3.7</td>
<td>57.2 ± 6.8</td>
<td>39.3 ± 5.1</td>
<td>51-60</td>
<td>32.1 ± 2.8</td>
</tr>
<tr>
<td>51-60</td>
<td>26.1 ± 0.8</td>
<td>52.4 ± 4.9</td>
<td>47.8 ± 7.3</td>
<td>53.6 ± 1.0</td>
<td></td>
<td>4.8 ± 2.6</td>
</tr>
</tbody>
</table>

Conclusion: The data illustrate an aging effect on semen characteristics and sexual behavior in aging men. However, although the semen quality declines with age, in this group of normospermic men, the semen parameters were still above normal levels according to WHO. However, it is possible that in cases of male factor infertility, this aging effect on semen quantity would be significant enough to impact on sperm function and the males’ ability to reproduce. This decline in sperm quantity and quality along with the decreasing sexual frequency could cumulatively have a negative effect on fecundity. It is suggested that the aging effect in males be taken into consideration when proposing normal standard values for semen characteristics in routine semen analysis as outlined by the WHO standards.

**P1/2 - 102**

**FAILURE OF PIASα EXPRESSION IN HUMAN TESTES OF MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA: POTENTIAL ETIOLOGIC FACTOR IN DESTRUCTIVE HUMAN SPERMATOGENESIS.**

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Using differential polymerase chain reaction (ddPCR) technique, we have previously identified an mRNA transcript that is not expressed in the testes of infertile men with pure Sertoli-cell only pattern but is expressed in testes of men with normal spermatogenesis. This mRNA transcript was identified to be PIASα based on full length cDNA cloning. Subsequent studies in rodent models have demonstrated that PIASα is expressed in germ cells (spermatogonia, spermatocytes and round spermatids). The objective of the present study was to evaluate the expression of PIASα in testicular tissues from infertile men with maturation arrest and hypospermatogenesis. Testicular biopsies were obtained from 16 non-obstructive azoospermic men with hypospermatogenesis (*n=5*), maturation arrest (*n=8*) and Sertoli-cell only pattern (*n=3*), and from one man with normal spermatogenesis. Northern analysis demonstrated absence of PIASα expression in 40% (2/5) of patients with hypospermatogenesis and sperm found on testicular sperm extraction (TESE). In addition, 50% (4/8) samples with maturation arrest had no PIASα mRNA detectable with Northern analysis. As expected, no samples (0/3) from testes with Sertoli-cell only pattern expressed PIASα. The absence of detectable PIASα mRNA levels, despite the presence of all germ cells on biopsy suggests a potential etiologic role of PIASα in the defective spermatogenesis for these patients. Genomic DNA analysis of patients with an absence of PIASα expression at the tissue level is planned to define whether PIASα mutations or deletions may be present as an etiologic factor in these infertile patients.

**P1/2 - 103**

**MALE INFERTILITY: IS AFRICA DIFFERENT?**

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Barrenness is traditionally blamed on the woman and although she might not even contribute to the infertility, she is the one affected most by the childlessness. This applies particularly to the woman in an infertile African couple. The question arose what the contribution of the man was to a couple's infertility in patients at two university clinics in South Africa. The results of the clinical history, physical examination (WHO, 1993) and semen analyses (WHO 1992) and first semen analyses of 2888 male patients were evaluated. Major findings included a high incidence of previous sexually transmitted diseases (30%), cigarette smoking (38%), obesity (33.5%) and azoospermia (9.3%). A significant male factor causing infertility was demonstrated in 40.1% of men. The seemingly unhealthy lifestyle of patients, reflected by the high incidence of obesity and cigarette smoking, is alarming. Sexually transmitted diseases are a major cause of infertility in both males and females. Greater public, social and cultural understanding be sought for the male role in infertility. An urgent and comprehensive approach to the health of the adolescent in developing countries is of paramount importance.

**P1/2 - 104**

**INHIBITION OF IN VITRO FERTILIZATION IN THE HAMSTER BY ANTIBODIES RAISED AGAINST THE RAT SPERM PROTEIN SP22.**


SP22, a rat sperm membrane protein that is highly-correlated with fertility, is being investigated as a biomarker of sperm function. Antibodies raised against SP22 have been shown to inhibit in vitro fertilization (IVF) in the rat, and localize to the equatorial segment of rat and hamster spermatozoa, suggesting a role for SP22 in fertilization. Herein, we examine whether SP22 antibodies inhibit IVF in the hamster. Affinity-purified, polyclonal sheep anti-recombinant SP22 (rSP22) was added to hamster sperm during the final hour of a 3 hour capacitation incubation. Sperm were then used to inseminate zona-intact (ZI) and zona-free (ZF) hamster oocytes. rSP22 Ig inhibited fertilization of ZI oocytes in a concentration-dependent manner, without apparent effect on sperm motility. Mean fertilization for 3 experiments was 95, 64*, 18*, and 6% for undiluted, 1:50, 1:10, and 1:5 Ig dilutions, respectively (* denotes p < 0.05). These results suggest that SP22 may function in sperm binding to and/or penetration of the zona pelucida. Mean fertilization of ZF oocytes was 98, 100, 92, and 53% across dilutions, and polyspermoy was 5.6, 5.6, 3.0, and 0.8 sperm/oocyte across dilutions. Thus, SP22 might also play a role in sperm-oolemma fusion; or this may be a non-specific effect of increased Ig. Taken together, these findings suggest that antibodies to SP22 can be used to evaluate SP22 expression in other species, supporting the concept that SP22 might serve as a useful biomarker of fertility in toxicology, or as a target in contraceptive studies, in animal models and humans. This abstract does not necessarily represent EPA policy.
Abstracts – Poster Session 1/2

P1/2 – 105
RECURRENT MISCARRIAGE: COULD THE MALE PARTNER BE RESPONSIBLE?
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Introduction: Early pregnancy loss is a common and distressing problem, especially for couples who experience recurrent miscarriage. In many cases the aetiology of recurrent miscarriage is never established, and much of the research to date has concentrated on causative factors in the female partner. It is now well documented within the infertility population that men with poor semen quality have high levels of spermatozoal DNA fragmentation and it has been suggested that this may be one cause of early embryo loss. The aim of this study was to examine the relationship between human spermatozoal DNA fragmentation and early pregnancy loss. Methods: Male partners of women attending a recurrent miscarriage clinic participated. Control subjects were recruited from a semen donor programme, all had living children and had suffered from no more than one miscarriage. Semen samples were assessed routinely using techniques described by WIGO (1999) The level of spermatozoal DNA fragmentation was quantified using the single cell gel electrophoresis (Comet) assay and the terminal deoxynucleotidyl transferase dUTP mediated nick end labelling (TUNEL) assay. Results: There were no differences in basic semen parameters between the patient and control groups. However differences in sperm DNA integrity were detected using both DNA damage assays. The median (IQR) percentage TUNEL positive sperm in the recurrent miscarriage group was 18.0 (14.1-21.6) and the control group 8.6 (4.7-13.0), (p<0.001). Conclusions: This data suggests that there may be a relationship between the genetic integrity of the male gamete and the problem of recurrent miscarriage. It emphasises the importance of further research into the role of the male partner in embryo loss.

P1/2 – 107
VARICOCELE ELEVATES REACTIVE OXYGEN SPECIES LEVELS AND REDUCES ANTIOXIDANT ACTIVITY OF INTERNAL SPERMATIC VEIN BLOOD OF INFERTILE MEN WITH VARICOCELE.
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The aim of this study is to investigate whether varicocele may affect reactive oxygen species levels or the antioxidant capacity of the internal spermatic blood of infertile patients with varicocele. Material and Methods: The study group consisted of sixty-eight infertile males, selected from patients scheduled for varicocelectomy. We estimated the levels of three ROS radicals (malondialdehyde, hydrogen peroxide, and nitric oxide) and five antioxidants (superoxide dismutase, catalase, glutathione peroxidase, vitamin C, and albumin), in blood drawn from antecubital vein and the internal spermatic vein at the time of varicocelectomy. We compared serum reactive oxygen species and antioxidants levels of blood drawn from the antecubital vein, with equivalent figures of blood drawn from the internal spermatic vein. Results: The levels of the three tested ROS radicals were significantly higher in internal spermatic vein blood compared to peripheral blood. The levels of four of the five tested anti-oxidants (SOD, Cat, GPx, and Vit C) were significantly lower in spermatic vein blood compared to peripheral blood, however no significant difference was observed in the level of albumen. Conclusion: Varicocele elevates reactive oxygen species levels and reduces antioxidant activity of internal spermatic vein blood of infertile men with varicocele.

P1/2 – 108
SOMATIC ACE ACTIVITY IN LEFT SPERMATIC VEIN SAMPLES OF PATIENTS WITH LEFT VARICOCELE AND ITS CORRELATION TO THE SPERMATOLOGIC PARAMETERS
R Asci, S Sarliuva, A Belit, R Boyolphelli, AF Yilmaz Departments of Urology and Biochemistry, Onkolozik Mayo University Samsun -TURKEY
OBJECTIVE: The ACE is found as two isozymes in the body. A somatic isozyme found in blood and several other tissues, including the epididymis, and a tissue-specific isozyme found only in developing spermatids and mature sperm. In this study we aimed to show the ACE activity in left spermatic vein blood samples of infertile patients with varicocele and its correlation to the spermatologic parameters. METHODS: Synchronously, the activity of ACE was determined in the peripheral and left spermatic veins blood samples from 31 infertile patients with left varicocele who underwent varicocelectomy. As a control, ACE activities were assessed in peripheral and left spermatic vein samples from 11 fertile patients without varicocele who underwent left inguinal herniography. The ACE activity was measured by colorimetric assay. Semen analyses of all patients were performed according to WHO criteria. RESULTS: The mean ACE activities were 60.3 ± 23.0 and 60.2 ± 23.2 pmol/ml/1 minute in peripheral and left spermatic vein serum samples from varicocele group, respectively. In the control group, the mean ACE activities of the peripheral and left spermatic vein samples also were 56.8 ± 17.1 and 56.5 ± 15.5 μmol/ml/1 minute. There were no significant differences between the ACE activities of peripheral and left spermatic vein blood samples from varicocele and control group. There was no statistical significant correlation between the spermatologic parameters and ACE activities of spermatic and peripheral vein in both of varicocele and control groups. CONCLUSION: The serum ACE activities of peripheral and left spermatic vein were very similar in the infertile patients with left varicocele. There was no correlation between the serum ACE activity of left spermatic vein and the spermatologic parameters in patients with varicocele suffered from infertility.
P1/2 – 109
Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are proinflammatory cytokines modulating leukocyte response related or unrelated to infertility. They can be detected in genital tract secretions. Varicocele has been associated to leukocytospermia in patients without infection. We investigated seminal levels of TNF-α, IL-6 and semen parameters in patients with left varicocele (V: n=52) and fertile donors (F: n=16). Nine V patients were reevaluated after surgery. Microbiological studies and antisperm antibodies were negative in all cases. Cytokines were determined by ELISA and values were expressed as median and range. TNF-α (pg/ml) differed significantly between V (40.0; 5.0 - 450.0) and F (13.0; 5.0-20.0; p<0.001). V with grade III and I or reduced left testis volume showed the highest levels of TNF-α. After varicocelectomy TNF-α in V (16; 0; 5.0-90.0) did not show significant differences with F (p=0.173). IL-6 (pg/ml) in V (43.0; 31.0-640.0) was not different to F (34.0; 15.0-65.0). However IL-6 correlated negatively with fructose and citric acid (r=-0.29;p=0.04 and r=-0.36;p=0.01; respectively) and positively with the number of peroxidase white blood cells (PMN) (r=0.47;p=0.0001). In V (with PMN>500,000/ml) IL-6 was higher than F (220.0; 6.0-640.0; p=0.0001) and correlated negatively with the HOS test (r=-0.655; p=0.017). High IL-6 levels were associated with changes in sperm integrity. Further studies will establish the utility of seminal TNF-α as a predictive marker of the outcome of varicocele, after surgery.

P1/2 – 110
The epididymal stage plays a pivotal role in sperm maturation. The entrance into the spermatozoa of carnisine complete their development, during this stage. On the other hand, it has been demonstrated that, in oligospermia and necropermia, the carnisine seminal plasma levels are lower than normal controls. It has been reported that, after surgical therapy for varicocele, treatment with carnisine, L-acetilcarinimine and FSH improves seminal parameters. Aim of this study was to evaluate the action of carnisine and L-acetilcarinimine treatment after varicocelectomy. 47 patients, after 1 month from varicocelectomy, were randomly divided in 2 groups: A (n=17) control group, B (n=30) treated for 3 months as follows: L-carnitine 1g. b.i.d. and L-acetilcarinimine 0.5g. b.i.d. "per os". The patients, taking in account their sperm count, were divided in 3 sub-sets: 1<10 millions/ml, 2(10-20 millions/ml), 3(>20 millions/ml). The sperm count, motility and viability have been studied. The motility has been evaluated by C.A.S.A. The statistical analyses has been performed by Newman and Keuls test. The motility (total, VCL and LIN) and viability, after treatment, significantly increased in the group A2 (p=0.005). In conclusion, on the basis of our data, it is possible suggest that the therapy with carnisine and L-acetilcarinimine, after surgical treatment of varicocele, is useful, above all, in average oligospermia, even if these preliminary results must be confirmed on a more conspicious cohort of patients.

P1/2 – 111
ULTRASTRUCTURAL CHANGES OF THE SPERMATIC VEINS IN VARICOCELE. A El-Kamshouushi, W Abdallah, Y.Kosha*, S.Helal*, Faculty of medicine, Alexandria University, Egypt.
There is a lack of knowledge of specific electron microscopic (EM) changes in the spermatic veins in varicocele. Ultrastructural study of spermatic veins may give important information on the etiopathogenesis of varicocele and its effects on male infertility. In this study, specimens of left spermatic veins were taken from 20 infertile and 10 fertile patients with varicocele during inguinal varicocelectomy and from 10 fertile control patients without varicocele undergoing inguinal herniorrhaphy. All specimens of spermatic veins were examined by electron microscope. Preoperative written consent was taken from all patients; and clinical examination, doppler US and semenograms were done. The results revealed only mild flattening of the endothelium in fertile group with varicocele. In infertile patients EM changes included apoptosis of the endothelial cells, interruption of internal elastic lamina and phentotypic changes of the smooth muscle fibres, with appearance of fibroblasts. Large amount of collagen and increased intercellular matrix. No EM abnormalities were detected in veins taken from normal control fertile patients. The EM changes are positively correlated with the fertility potential of patients and abnormalities in semenograms. The results of this study indicated that specific EM changes may be present in the spermatic veins in varicocele. Progressive apoptosis of spermatic veins may lead to vascular dilatation and varicocele formation with resulting infertility. We suggest that unpredictable beneficial effects of varicocelectomy in some cases of infertility may be due to associated intratesticular venous system damage similar to that detected in spermatic veins in this study.

P1/2 – 112
Introduction: Varicocele is found to be the most common cause among the diseases associated with male infertility. With the development and systematic utilization of color Doppler examination, the ratio of the bilateral varicocele has decisively grown. Materiel and methods: At 81 bilateral varicocele patients the diagnostic exploration proceeding surgery has been carried out in a previously determined protocol (palpation, color Doppler, spermogram, genetic exams). The first step is bilateral testicular biopsy with scrotal exploration. The quantitative and qualitative spermagogenesis were analyzed in 4 biopic samples. The microsurghical intervention is performed on both sides with Goldstein technique (subinguinal mini-incision microsurgical varicocelectomy). Results: 1. Existence of spermagogenesis in original azospermic group 24/81 and the ratio of positive to negative sample was followed: in one testis 9/24 (37.5%) in 5 cases (20%) only on site of the testis. In both testes 15/24 (62.5%); 1-1 site 6, 3 sites 7. 4 sites: 2 (only 8%). 2. The ratio of azospermia before correction was 30% (24/81). This ratio fell after the microsurgical intervention to 20% (16/81). The global improvement of semen quality in original azospermic patients was 66% (16/24) after microsurgical correction. A statistical analysis of the pre- and postoperative semen data was compared by Wilcoxon matched pairs test. The medians were statistically significant for all parameters (count, motility, normal morpholgy), and p value is smaller than 0.05. The improvement with manzutus arrest was 56% and without it was 87%. Conclusion: Testis biopsy with microsurgical correction enriches diagnostics, and helps in judgment of possible success. In case of assist ed reproductive technique, testis biopsy with microsurgical correction makes possible to repetitively probe the most suitable and most qualitative tests site found in diagnosis.
Abstracts – Poster Session 1/2

P1/2 – 113
THE GONADOTROPIN RELEASING HORMONE (GnRH) STIMULATION TEST MAY PREDICT PREGNANCY OUTCOME AFTER MICROSURGICAL VARICOCELECTOMY
Marc Anthony Fischer, Khaled M. Khamei, Keith Jarvi, and Armand Zini. Toronto, Ontario, Canada (Presented by Dr. Fischer).
INTRODUCTION AND OBJECTIVES: Varicocelectomy can improve sperm quality in 70-80% of treated patients. There are no reliable factors that are predictive of successful varicocelectomy. This study is to examined the potential predictive value of a GnRH stimulation test in infertile men undergoing microsurgical varicocelectomy.
METHODS: The records of 49 infertile couples in whom the man underwent microsurgical varicocelectomy between September 1998 and June 2000 were evaluated retrospectively. All of these men underwent a GnRH stimulation test prior to their surgery. Data on semen parameters, pregnancy rates and FSH response to GnRH stimulation were recorded.
RESULTS: Post-operative sperm concentration increased significantly compared to pre-varicocelectomy (18.6 ± 6.8 vs. 26.1 ± 26.0 x 10^6 per ml, P<.05). Overall, 37% (18/49) of the couples achieved a spontaneous pregnancy at a mean of 16 months follow-up (range 12 - 20 months). In our study population, the median FSH rise at 120 minutes following GnRH administration was 1.5X baseline (range 1.1 to 5.0X). We observed a higher pregnancy rate in couples in whom the man's FSH response to GnRH stimulation was greater than 1.5X compared to those in whom the FSH response was less than 1.5X (45 vs. 9%, respectively. P=0.08).
CONCLUSIONS: Our preliminary data suggest that in couples in whom the man undergoes varicocelectomy for treatment of varicocele, the FSH response to GnRH stimulation may be predictive of unassisted pregnancy outcome in this population. Additional studies are needed to validate these initial results.

P1/2 – 114
THE INFLUENCE OF UNILATERAL AND BILATERAL LAPAROSCOPIC VARICOCELECTOMY ON PARAMETERS OF FERTILITY IN 290 MEN
J. Houk, M. Fricklowski. St. Horak Medical University Dept. of Urology and Andrology, Zabrze, Poland.
In the years 1995-2000 transperitoneal laparoscopic varicocelectomy was performed in 290 men with unilateral (group I-192 men, 66.2%) and bilateral varicoceles (group II-98 men, 33.8%). Surgical intervention was carried out by the two methods, ligation of internal spermatic vessels and artery sparing technique. Pre- and postoperative diagnosis was based on physical examination, standard scrotal sonography with color Doppler ultrasound and semen analysis in accordance with WHO standards. The treatments sufficiency was assessed in two age groups: A(18-30) and B(31-42) years old, respectively. Gr.I before op. after Gr.II before op. after count[x10^6/ml]19.5±10.5234.58±14.1217.32±9.22 55.4±21.46 motility[%] 19.06±9.09 34.70±15.32 19.14±10.20 56.71±22.82 normal forms[%] 22.36±9.18 53.86±22.88 20.82±8.42 61.24±21.24 Pregnancy rate 23.1 % 40.6% Gr.A before op. after Gr.B before op. after count[x10^6/ml]20.1±10.20 58.69±22.34 17.4±8.70 32.84±8.41 motility[%] 25.93±12.86 57.62±13.06 21.23±12.86 28.09±7.06 normal forms[%] 21.81±7.96 54.81±22.36 20.61±7.87 60.33±21.26
Pregnancy rate 36.4%18.6% Bilateral laparoscopic varicocelectomy remarkably improves sperm count, motility and pregnancy rate nearly two times in comparison to unilateral varicocelectomy. Laparoscopic varicocelectomy independently the location of varicocele, significantly improved the semen quality and pregnancy rate in wires of men up to 30 years old.

P1/2 – 115
TOTAL ANTIOXIDANT CAPACITY IN SEMINAL FLUID OF VARICOCELE PATIENTS:CORRELATION WITH SPERM MOTILITY.
1A. Marcini, E. Menczi, T. D밀라르, 1A. Bianchi, 2E. Giacchi, L De Marinis, L. Jensen. Institutes of 1Endocrinology, 2Biochemistry, Center for Study and Research on Natural Family Planning, Catholic University of the Sacred Heart, Rome, Italy.
Mammalian spermatozoa produce reactive oxygen species (ROS) implicated in hyperactivated motility and acrosome reaction, but they are also sensitive to ROS-induced damage. Varicocele (VAR) patients present an interesting model since they exhibit an augmented ROS generation and high levels of nitric oxide, by itself related to ROS generation. To further explore a possible molecular defect in VAR, we evaluated total antioxidant capacity in a group of VAR patients (10 oligospermic and 14 normospermic). Total antioxidant capacity was measured in seminal plasma aliquots using myoglobin as a source of radicals, which interact with a chromogen 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), whose radical cation is spectrophotometrically detectable. Antioxidants induce a lag time in the accumulation of ABTS cation proportion to their concentration. Trolox, a water soluble tocopherol analogue, was used as a reference standard and antioxidant capacity was expressed as umolar Trolox equivalent. The normospermic VAR patients (meansSEM 92.6±5.2 x10^6 cells/ml) showed Lag values (95.3±7.4 sec) not significantly different from those found in oligospermic patients (12.3±2.1x10^6 cells/ml). Lag 95.1±20.4 sec). No difference was noted in sperm motility (22.4±4.3 vs 17.7±3.3% of progressive cells respectively). A significant correlation was present when plotting Lag values and motility in normospermic (r=0.65; p<0.01), but not in oligospermic patients. These findings suggest the potential protective role of antioxidant in sperm motility, when it is not present a testicular damage. Further studies can clarify the role of antioxidant in VAR patients.

P1/2 – 116
SCROTAL TEMPERATURE AND VARICOCELECTOMY: VARICOCELE OR VARICOCELES?
Introduction: Relationships between scrotal temperature and varicocelectomy are rather scarce. The aim of the present study was to evaluate the effect of varicocelectomy on scrotal temperature. Methods: 66 non azospermic infertile patients with a clinical varicocele were submitted before and 3 months after surgery to the following measures: sperm characteristics, seric hormonal gonadostrophins and testosterone, testis volumes, and right and left scrotal temperatures. Results: A uni or bilateral scrotal hyperthermia was recorded in 45.5% of the patients. In the group of patients with a normothermic varicocele (n = 36) before surgery, 4 (11%) had a post-surgery scrotal hyperthermia. In the group of patients with an hyperthermic varicocele (n = 30) before surgery, only 2 had still a scrotal hyperthermia after surgery (6.7%). As concerns sperm characteristics after surgery, mean semen volume, sperm count, sperm motility and vitality were significantly increased in the presurgical normothermic varicocele group, while only mean sperm vitality was significantly increased in the presurgical scrotal hyperthermic varicocele group. Conclusions: Only one varicocele out of two is associated with scrotal hyperthermia. The absence of improvement in semen characteristics 3 months after surgery in the presurgical scrotal hyperthermic group could be explained by 1) a time longer than 3 months is required for a sperm improvement, as already observed in testicular induced hyperthermia in humans 2) varicocele could be only a secondary alteration to an other factor, so that varicocelectomy is without effect on sperm characteristics.
P1/2 – 117
TOTAL ANTIOXIDANT CAPACITY IN SEMINAL FLUID OF VARICOCELE PATIENTS: CORRELATION WITH HORMONE PATTERN.
1D. Milardi*, 1A. Mancini, 2E. Meucci*, 1A. Bianchi*, 2E. Giacchi*, 3L. Jensen*, 1L. De Marinis*. 1Institutes of 1Endocrinology, 2Biochemistry; 3Center for Study and Research on Natural Family Planning, Catholic University of the Sacred Heart, Rome, Italy

Human sperm cells are sensitive to ROS-induced damage and possess major antioxidant defense against ROS, including catalase, superoxide dismutase and glutathione peroxidase, whose effectiveness is limited by their low concentration and cellular distribution. Seminal plasma is well endowed with antioxidant buffer capacity. It is unclear if the antioxidant capacity is under the direct control of pituitary gonadal axis. Previous studies have reported the correlation between glutathione and FSH concentration. To further explore this topic we have evaluated total antioxidant capacity in a group of patients affected by varicocele (10 oligospermic and 14 normospermic). Total antioxidant capacity was measured in seminal plasma aliquots using myoglobin as a source of radicals, which interact with a chromogen 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS), whose radical cation is spectrophotometrically detectable. Antioxidants induce a lag time in the accumulation of ABTS cation proportional to their concentration. Trolox, a water soluble tocopherol analogue, was used as a reference standard and antioxidant capacity was expressed as μmol Trolox equivalent. The lag phase correlated significantly in the group of varicocele patients with the serum FSH levels (r=0.69; p<0.05), but the correlation was not present with testosterone levels. These findings suggest the dependence of total antioxidant capacity by the gonadotropin secretory in varicocele patients. Further studies can clarify the role of pituitary gonadal axis in the control of antioxidant species involved and its effective physiological role.

P1/2 – 119
TESTICULAR VOLUME IN INFERTILE VARICOCELE PATIENTS WITH NORMAL AND ABNORMAL FOLLICLE-STIMULATING HORMONE

Serum follicle-stimulating hormone (FSH) are especially sensitive to diffuse testicular injury, since an increase in serum concentrations attends any of the testicular insults, particularly those involving the seminiferous tubules. Also, it is well known that testicular volume correlates well with semen quality and infertility. We evaluated the levels of luteinizing hormone (LH), total testosterone, and testicular volume in infertile varicocele patients with normal and abnormal serum FSH levels. The records of 27 infertile patients with varicocele in the left testicle were evaluated from January 1999 to September 2000. In all patients serum hormone levels as well as testicular volume assessed with calipers were evaluated. Patients were divided into two groups according to FSH levels: group A (FSH < 12 mIU/mL; n = 22) and group B (FSH > 12 mIU/mL; n = 5). No differences were seen in the mean age of the patients in groups A and B: 29.08 ± 7.2 and 26 ± 3.2, respectively (P > 0.05). FSH levels were significantly different between the two groups (5 ± 2.9 and 20.74 ± 9.8; P = 0.01). LH levels were higher in group B (13.12 ± 8.19) compared to group A (5.42 ± 3.61) (P = 0.02). No differences were seen in the right testicle volume in group A (19.43 ± 7.8) compared to group B (16.59 ± 14.38) (P > 0.05). The left testicle was bigger in group A patients (17.7 ± 8.6) compared to group B (11.7 ± 9.5) (P = 0.04). Infertile men with varicocele and high FSH levels have small left testicles compared to left testicles of varicocele patients with normal FSH levels.

P1/2 – 120
SEMINAL REACTIVE OXYGEN SPECIES (ROS) IN VARICOCELE PATIENTS: PRELIMINARY RESULTS DEMONSTRATE TREND OF INCREASE ROS LEVELS WITH VARICOCELE GRADE, NOT TESTIS SIZE.

 Ipsilateral testicular atrophy is associated with decreased total motile sperm counts in infertile men with clinical varicoceles, particularly large varicoceles. Increased levels of ROS are also associated with clinical varicoceles; however, its correlation with testis size and varicocele grade is unknown.

We retrospectively reviewed the ROS levels of 26 men who presented with unilateral left varicoceles. Varicoceles were graded and testis size determined by caliper by an experienced examiner (AJT). Testicular volume was calculated by 0.52 X length X width X depth. ROS levels were determined by a chemiluminescence assay.

Nonparametric correlations with ROS levels showed no significance with left testis size (p=0.98); however, there was a trend of increased ROS levels with grade of the varicocele (p=0.10). There was no difference in ROS based on a comparison group classified by left testis volume (p=0.72) or a difference in right and left testis volume (p=0.84); however, a trend was again seen comparing left grade 1 versus grade 2-3 (p=0.12).

Although a larger sample size will be needed to determine if a true relationship exists, increased ROS levels may be predictor of varicocele-induced infertility.
**Abstracts – Poster Session 1/2**

**P1/2 – 121**

**Efficacy of subinguinal varicocelectomy on semen parameters and pregnancy in men with severe oligozoospermia**

Keiji Ogura, Jun Watanabe, Kazutoshi Okubo, Yoichi Arai
Department of Urology, Hamamatsu Rosai Hospital and Kurashiki Cenyral Hospital, Hamamatsu and Kurashiki, Japan.

**Objectives:** To characterize outcome following varicocele repair in men with severe oligozoospermia, prospective non-randomized study to assess the improvement of sperm parameters and pregnancy rates was performed.

**Materials and Methods:** Patients with a palpable varicocele tests and severe oligozoospermia of a sperm density of less than 5 million/ml per ejaculate was enrolled. All men underwent a testicular artery-sparing technique of subinguinal varicocelectomy without delivery of the tests using a roupe or microscope. Eleven (17%) of 65 men who underwent a varicocelectomy during April 1995 to March 1998 met the enrollment criteria. Enrollment criteria for men who have yet to contribute to a pregnancy included a minimum of 3 months of follow-up and two semen analysis. Ten (91%) underwent unilateral left-sided procedure, and one (9%) underwent bilateral procedures. The varicocele was considered grade 3 in 6 men, grade 2 in 4 men and grade 1 in one.

**Results:** Sperm density and the total number of motile sperm increased from 2.3 million/ml and 3.4 million to 16.8 and 22.3 (p=0.0087 and p=0.0225). Six men (55%) of 11 achieved pregnancies at 9.2 months after varicocelectomy.

**Conclusions:** Varicocele repair should be considered for men with severe oligozoospermia before performing assisted reproduction technique.

**P1/2 – 123**

**HORMONE LEVELS AND TESTICULAR VOLUME IN FERTILE MEN WITH VARICOCELES, INFERTILE MEN WITH VARICOCELES, AND FERTILE CONTROLS WITHOUT VARICOCELES**

Divisa de Clinica Urologica do Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo.

Testicular alterations associated with the varicocele are well documented. Also, altered testicular steroidogenesis as a mechanism by which the varicocele exerts its deleterious effects on spermatogenesis remains unclear. We evaluated the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, and testicular volume in fertile and infertile men with varicoceles, and fertile controls without varicoceles. The records of 27 infertile patients with varicocele and 70 controls with and without varicoceles were assessed from January 1999 to November 2000. In all patients serum hormone levels as well as testicular volume assessed with calipers were evaluated. Patients were divided into three groups: group A (fertile men with varicocele; n = 21), group B (infertile men with varicocele; n = 27), and group C (fertile men without varicoceles; n = 49). No differences were seen in the mean age and testosterone levels across the three groups (P > 0.05). FSH levels were significantly higher in group B (7.8 ± 7.6) compared to groups A (2.9 ± 1.38) and C (3.43 ± 2) (P < 0.05). Also LH levels were higher in group B (6.8 ± 5.4) compared to patients in groups A (3.2 ± 1.9) and C (3.4 ± 1.74) (P < 0.05). The right testicle was smaller in group B (19.9 ± 9) compared to group A (24.4 ± 8.6) and C (25.6 ± 8.5) (P < 0.05). Also, the left testicle was smaller in group B (16.6 ± 8.9) compared to groups A (21 ± 7.8) and C (23.4 ± 8.3) (P < 0.05).Infertile patients with varicocele have higher levels of FSH and LH and smaller testes compared to controls with and without varicoceles.

**P1/2 – 122**

**VARICOCELE REPAIR IMPROVES SEMEN PARAMETERS IN AZOOSPERMIC MEN WITH TESTICULAR FAILURE IRRESPECTIVE OF TESTICULAR HISTOLOGY**

FF. Pasqualotto*, A.M. Lucon*, J. Hallali, L.B. Saldanha*, P.M. Gôes*, J.R. Colombo*, S. Arap*, University of Sao Paulo Medical School, Brazil.

There is clinical evidence to suggest that spermatogenesis was damaged or failing tests may vary geographically, resulting in focal areas or "patches" of sperm production within an organ largely devoid of germ cells. Therefore, the role of a single large testis biopsy to predict the semen analysis improvement as well as pregnancy outcome in patients undergoing varicocelectomy may be useless. We sought to correlate the testicular histology patterns from a group of azoospermic men to treatment outcome after varicocele repair. The records of 9 azoospermic men who underwent testis biopsy and microscopic subinguinal repair of clinical varicocele from July 1999 to April 2000 were reviewed. All patients had at least 2 semen analysis taken before the surgery showing azoospermia and at least 1 semen analysis at 6 months post operatively. All biopsies were analyzed by an experienced pathologist. Hypospermatogenesis (HYPO) was identified in 1 man, maturation arrest (MA), and Sertoli cell only (SCO) in 3 men. Induction of spermatogenesis was achieved in 4 men (44.4%). Of these 4 men, 3 had a SCO syndrome and 1 had a MA. The sperm concentration as well as sperm motility achieved in the patients with SCO were 7.9 million/ml, 7.5%, 2.5 million/ml, 35%, and 3.8 million/ml, 32%, respectively. In the patient with MA, the sperm concentration was 3.5 million/ml, and 24% sperm motility. No pregnancies were reported in the follow-up. A single testis biopsy showing SCO may not reflect the overall testis histology but just a focal area. Therefore, azoospermic patients with SCO in a single large testis biopsy may have an improvement in a semen analysis following varicocelectomy.

**P1/2 – 124**

**INCIDENCE OF VARICOCELE IN CHILDREN AND ADOLESCENTS**

KH Rha, BH Kim, SW Han, MS Lee, Department of Urology, Yonsei University, Seoul, Korea.

To elucidate the current incidence of varicocele in an unselected Korean population, 4,271 Korean school boys aged 6-17 years was investigated. The study population comprised more than 95% (4271/4403) of all boys in the respective age in a county with a total population of 61,820(29,840 males and 31,980 females). The study was carried out by a single urologist (B.H.K.) from March to June 2000. No case of varicocele was encountered in 1,233 boys between 6 to 9 years, while the incidence in the 3,038 boys aged 10 to 17 years was 9.24% (281 boys). There was a peak in varicocele incidence from age 12 (18.48%); 61 (330), 13 (13.56%); 48 (354) and 14 (16.56%); 57 (355). The proportion of varicocele grades were grade I 21.36% (60/281), grade II 45.5% (129/281) and grade III 36.5% (102/281). There were 4 bilateral varicoceles. Other abnormalities found were 21 cryptorchidism (0.49%); 21/4271 and 19 hydroceles (0.44%); 19/4271. In 34 older boys (ages between 15-17) with varicocele, semen analysis was performed and only one boy had subnormal sperm concentration (14 million/ml). Our finding of peaking at ages 12 to 14 was parallel with the old English(Horner JS, Medical Officer 104: 377, 1960) and Danish(Oster I, Scand J Urol Nephrol 5: 27, 1971) reports, but the our incidence was substantially lower than previous reports.
P1/2 – 125

MICROVASCULAR TESTICULAR BLOOD FLOW AS EVALUATED BY LASER DOPPLER FLOWMETRY AFTER THE SURGICAL INDUCTION OF VARICOCELE

N Salama*, A Bergh† and JE Damberg†. From Departments of Urology, Alexandria, Egypt and Urology and Andrology* and Pathology† Umeå University, Umeå, Sweden

This study was conducted to evaluate the changes in microvascular testicular blood flow (microvascular TBF) which may be associated with varicocele. The pathology was developed through partial ligation of left renal vein in 4 groups of rats. Controls of each group underwent sham surgery. It was observed that microvascular TBF dropped and its vasomotion became inhibited once the left renal vein was partially ligated as studied by laser Doppler flowmetry (LDF). Four to five minutes later blood flow rose to the pre-treatment level and that of controls. Vasomotion reappeared with a uniform frequency and amplitude. At different periods of varicocele bearing (1w, 3w, 6w & 14w) microvascular TBF was re-evaluated. Vasomotion appeared intact without any abnormalities in the four groups. To examine the response of testicular microvasculature after surgical creation of the varicocele to pharmacological stimulation another group of animals received human choric gonadotropin (hCG) treatment 6 w after varicocele creation. Vasomotion was inhibited in these animals. In conclusion, LDF is a suitable tool to evaluate microvascular TBF in vivo. It can detect acute changes in blood flow which may happen at surgical induction of varicocele. In addition, the vasculature of the testis with a surgically induced varicocele can still respond to hCG stimulation.

P1/2 – 126

THE CHANGES IN TESTICULAR VASCULAR PERMEABILITY DURING PROGRESSION OF THE EXPERIMENTAL VARICOCELE

N Salama*, A Bergh† and JE Damberg†. From Departments of Urology, Alexandria, Egypt and Urology and Andrology* and Pathology† Umeå University, Umeå, Sweden

The vascular permeability of testicular capillaries was studied during the progressive course of experimental varicocele (EV). The pathology was developed through partial ligation of left renal vein in 4 groups of rats. Controls of each group were subjected to sham surgery. At different periods of varicocele bearing, (1, 3, 6, 14 w) animals' testes per one of the study groups were exiripated and weighed where those of (1 & 3 w) groups were found to be significantly heavier (p<0.05) than their controls. The volume density percentages of polymorphnuclear leukocytes (PNL) per testicular blood vessels were also estimated in both testes and were significantly higher in each study group than in those of its controls. However, these percentages showed gradual significant decreases as the duration of varicocele bearing gradually increased. To further verify these findings another group of animals received human choric gonadotrophin (hCG) treatment 6 w after varicocele creation. Histopathological sections of these animals revealed more accumulation of the PNL. Therefore, the present results suggest that EV may induce an increase in testicular vascular permeability which then decreases gradually with time. It is supposed that this may be accompanied by parallel changes in the rate of formation of the testicular interstitial fluid. The study also showed that the vasculature of the testis with EV can still respond to hCG.

P1/2 – 127

THE INCIDENCE, SPECIFICITY AND SENSITIVITY OF CLINICAL EXAM FOR THE DIAGNOSIS OF VARICOCELE COMPAARED TO DUPLEX DOPPLER ULTRASOUND O Shah*, S Telegrafi*, AR McCullough, New York Univ. School of Med. The use of ultrasound in the diagnosis of male infertility is controversial. To evaluate the application of routine testicular Doppler we compared ultrasound findings in infertile men undergoing testicular dopplers vs non-infertile men. During 18 month, 231 men underwent testicular ultrasound as part of their urological evaluation (122 infertile-I, and 109 non-infertile-NI). All ultrasound were performed by the same ultrasonographer with an Acuson Advanced Aspen system with 7 to 10 mgHz probes.

Table 1. Clinical Diagnoses (CI Dx) expressed as %

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Bilat. varic.</th>
<th>Left Varic.</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>24</td>
<td>42</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>Non-Infertility</td>
<td>37</td>
<td>12</td>
<td>20</td>
<td>39</td>
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</tbody>
</table>

Table 2. Varicoceles in Infertile and non-infertile males

<table>
<thead>
<tr>
<th></th>
<th>Mean Age</th>
<th>Bilat. varic.</th>
<th>Left Varic.</th>
<th>Right Varic.</th>
</tr>
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<tbody>
<tr>
<td>NI (n=108)</td>
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<td></td>
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<tr>
<td>Ultras. Diag.</td>
<td>19</td>
<td>23</td>
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<tr>
<td>CI Dx</td>
<td>12</td>
<td>20</td>
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<tr>
<td>I (n=122)</td>
<td>36.6</td>
<td></td>
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<tr>
<td>Ultras. Diag.</td>
<td>30</td>
<td>42</td>
<td>1</td>
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<tr>
<td>CI Dx</td>
<td>43</td>
<td>19</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Sensitivity of clinical diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI BV</td>
<td>9/19 (47)</td>
<td>25/27 (93)</td>
</tr>
<tr>
<td>LV</td>
<td>17/23 (74)</td>
<td>22/23 (96)</td>
</tr>
<tr>
<td>I</td>
<td>21/30 (70)</td>
<td>30/43 (70)</td>
</tr>
<tr>
<td>LV</td>
<td>13/42 (31)</td>
<td>30/31 (97)</td>
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Sonographically diagnosed varicoceles in I patients was approximately 60% vs 43% in NI patients. 32% and 17% of I and NI patients were incorrectly diagnosed. Thus, the routine Doppler analysis of infertility is useful in accurately identifying and diagnosing varicoceles.

P1/2 – 128

SURGICAL TREATMENT OF VARICOCELE – CONTROVERSIAL PROBLEM. A LONG-TERM FOLLOW-UP STUDY

GA Szmyczynski, Department of Clinical Andrology, The Ludwik Rydygier Medical University in Bydgoszcz, Poland

Analysed material was a population of 356 infertile patients (mean age 28.89 ±SD 4.55) divided into three groups: I - 302 patients; II - 41 patients; and III - 13 patients in whom after the first surgery and control phlebography varicocele persistent were found out. Surgery (high liga-tion) was done in the years 1981 - 1990 and the time since infertility had been recognized ranged from 12 months to 17 years. In group I analysis of fresh semen - 1121 specimens, and morphological examination of dried smears on 1103 slides were done; group II - 204 and 201; group III - 84 and 82, respectively. In three groups 1409 fresh and 1386 dried smears were statisctically analyzed. Every twelfth weeks (±3 weeks) after the surgery patients were systematically controlled by seminal analysis. 18 months later, detailed data were carefully reanalyzed based on both clinical investigations and additional tests. Data were verified after 6 - 15 years, by relevant forms sent to available patients. Achieved results suggest that high ligation of varicocele is an effective method of male infertility treatment if indications for surgery are individualized and standardized, critical semen examination before and after the operation is well planned and realized in advance. No reason was found for surgery in every case of such vein patholgy - varicocele, but in individual cases surgery is advisable. No improvement in semen analysis within 18 months after the surgery proves the necessity for X-rays control to set up the strategy of next step: resurgery, embolization or sclerotization during X-rays examination or ART. In dubious cases (particularly younger men) conservative treatment is recommended as a widened diagnostic procedure. If after successful surgery, radiologically proved (no reflux), there is no improvement in semen value the patient should be qualified for ART. Nevertheless, in every case of reanalysis both partners should undergo evaluation of fertility potential.
P1/2 - 129
IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NITRIC OXIDE SYNTHASE (NOS) ISOENZYMES IN HUMAN VARICOCELE AND EXPERIMENTAL RAT VARICOCELL.
James K. Tan, Amer K.*, E.A. Ling* Department of Surgery and Anatomy, National University Hospital, Singapore.

Introduction: Nitric oxide (NO) has recently been implicated in the pathophysiology of sperm dysfunction in varicoceles. The aim of the study is to characterize the NOS isoenzymes in the wall of human varicocele as well as the experimental left varicocele (ELV) and testes of the rat. Method: 20 rats underwent creation of ELV by partially ligating the left renal vein. 3 rats underwent sham operation. All rats were sacrificed after 4 weeks and examined for presence of varicocele. The dilated spermatic veins and testes of 6 rats with well developed ELV and 3 controls were harvested and studied using immunohistochemical technique. The NO synthase (NOS) iso-forms were detected by monoclonal antibodies and characterized in the various layers of the veins and the rat testes. Meanwhile 18 human varicoceles and 2 normal testicular veins were also studied. The NOS density in the human varicocele was compared to the clinical grade of varicocele. Result: Endothelial NOS (eNOS) and inducible NOS (iNOS) but not neuronal NOS (nNOS) were found in the media and adventitial layers of both the rat and human varicocele. Both eNOS and iNOS were weakly and inconsistently present in the controls. The rat testes showed very low density of iNOS and eNOS. The density of both eNOS and iNOS in the human varicocele could not be correlated with the clinical grade of the varicoceles. Conclusion: There is expression of iNOS and eNOS in the wall of the rat and human varicocele which is not seen in the rat testes. NO may exert a post-testicular local effect within and around the spermatic vein which then impairs sperm function.

P1/2 - 130
MICRO-MEASUREMENTS OF MICROCIRCULATION PERFUSION AND THERMAL PARAMETERS IN HUMAN TESTES DURING MICROSURGERY OF VARICOCELE.
Joseph TRITTO, Department of Urology, Division of Surgical Andrology, Saint-Louis Hospital, Paris; Marie-Odile NORTH, Service d'Histologie-Embryologie-Cytogenetique, Hopital Necker, Paris; Andre DITTMAR, Biomedical Sensors Systems Dept., CNRS LPM, INSIA, Lyon – France

Male reproductive organs are specifically thermo-regulated (A.W.Zergometti, Adv. Exp. Med. Biol., 286, 199, 1991). Micro-measurements of microcirculation and thermal parameters were applied for diagnostic purposes in male infertility (A.Dittmar, J.Tritto et al., IEEE EMBS, 15, 992, 1993). Active thermal microsensors, measuring in real-time thermal conductivity, diffusivity and microperfusion are implanted into the gonads with microsurgery to quantify the thermal responsiveness of the microcirculatory environment. The thermal diffusion microprobes (TDP) use a self-heated thermistor to measure absolute perfusion continuously and are validated at low flows with the microsphere technique (C.T.Martin, H.F.Bowman, Med.Biol. Eng. Comput., 38, 319, 2000). The micro-measurements are compared in vivo to Laser Doppler Flowmetry (LDF) on vasomotion and Optic Video Micro-Capillaroscopy (OPVMC) on microvascular patterns. In thermodynamically-impaired testes associated to clinical infertility, microsurgical correction of the bilateral varicocele is realized, monitoring in parallel the thermal behaviour of the gonads. Tissue blood flow and thermal conductivity are measured during and after microsurgery; the parameters are implemented on the fractal thermodynamic model of the human tests for simulation (J.Tritto et al., IFMBE, 35, 598, 1997). Minimally invasive microtechnologies (MIMT) assisted by micro-mechatronic tools (MiMET), applied to the thermoregulation of the human tests during surgery for male infertility, represent advanced methodologies to support new ARIs.

P1/2 - 131
DETERIORISATION OF SPERM PARAMETERS IN MEN WITH UNTREATED VARICOCELE
I. Vukovic*, N. Bojanic*, N.Lalic*, S. Micic, Clinic of Urology, Belgrade, Yugoslavia

Varicocele has been repeatedly implicated as a cause of male infertility. The question of whether varicocele represents a deteriorative process that is evidenced and worsens over time has been addressed by various studies using animals followed at progressive intervals. Lipshultz and Corriere found sperm densities decreased by 50% in older men with varicocele when compared with younger men. The aim of the study is to report observation of sperm parameter in men with untreated varicocele over time. Sperm parameters in 19 men with varicocele were obtained at initial visit and re-evaluated at 10-98 months interval because of persistent barren marriage. All men have left sided varicocele (grade I: 3, grade II: 12, grade III: 4). Initial evaluation revealed normal sperm parameters. Deterioration in sperm count and motility were found to be significant. Sperm count which was normal in 19 patients, become abnormal in 14 (74%). Also progressive motility was initially normal in 12,9 of which (75%) became abnormal. This data suggest that testicular deterioration in men with varicocele may in a progressive manner. We believe that in these cases, normal sperm parameters should not routinely be expected to stay normal and close follow-up is needed.

P1/2 - 132
THE EFFECT OF PROGESTINS ON SERUM TESTOSTERONE, AGGRESSION AND SEMEN PRODUCTION IN GERENUK (LITOCRANUS WALLERI WALLERI).
LM Penfield I, E Plotka* and SB Citino*, White Oak Conservation Center1, Yulee, FL and Marshfield, WI2

The use of progestins to suppress endogenous testosterone production in order to reduce aggressive behavior and prevent semen production was studied in gerenuk. Five male gerenuk (aged 20 mo - 3 yr), housed as a bachelor group, were treated with 30 monthly injections of medroxyprogesterone acetate (MP, 2.5mg/kg), followed by a melangestoral acetate implant (MGA; 0.3g/kg) for 2 months. Blood samples, collected monthly, were assayed for serum testosterone using a testosterone enzyme-linked immunosassay validated for gerenuk. Quantitative behavioral data were collected for 30 min, 3/week starting one month before treatment. Body weight, testes volume, and semen and sperm traits were measured before treatment, after MP treatment, and after MGA treatment. Results showed lower (P<0.05) mean serum testosterone concentrations after MP (0.05 ng/ml) and MGA (0.04 ng/ml) treatment compared to pre-treatment values (0.42 ng/ml) in 4/5 gerenuk. The remaining sub-adult gerenuk had low testosterone initially (0.05 ng/ml) that did not decrease further with treatment (0.05 and 0.08 ng/ml for MP and MGA, respectively). The mean incidence of combined aggressive/ dominant behaviors (homing, sparring, supplanting, threat) was unchanged before and after treatment, as were body weight, total numbers of spermatozoa produced (vol 0 conce), percent motility and percent normal spermatozoa. However, mean testes volume decreased (P<0.05) after MGA treatment (11.96 cm3 vs 10.53 cm3). Elevations in hepatic enzymes and bile acids were seen in 3/5 animals after MGA treatment. Results show that reducing serum testosterone does not reduce aggressive behavior or semen production in bachelor gerenuk.
P1/2 – 133

ACHIEVING AZOOSPERMIA BY INJECTIONS OF TESTOSTERONE UNDECANOATE ONLY OR COMBINED WITH DEPOT MEDROXYPROGESTERONE ACETATE IN INDONESIAN MEN (JAKARTA CENTRE STUDY)

Nurman Moeloek, Dwi Ari Pujianto and Rulliana Agustin.Andrology Unit, Department of Medical Biology, Faculty of Medicine, University of Indonesia. Jakarta, Indonesia

The effects of long acting testosterone, testosterone undecanoate (TU) only, or combined with depot-medroxyprogesterone acetate (DMPA) were investigated to determine the suppression of sperm production in achieving azoospermia in Indonesian men. Twenty men were divided into two groups, the first group injected with 500 mg TU at intervals of 6 weeks (n=10), and the second group injected with 500 mg TU also at intervals of 6 weeks and 300 mg DMPA at intervals of 12 weeks (n=10). Six weeks after first shot injections sperm concentrations decreased from 43.38±4.57 million/ml (mean±SEM) to 24.26±8.05 million/ml, and 10% men achieved azoospermia in the TU group. The sperm concentrations decreased sharply from 36.45±5.52 million/ml to 0.82±0.34 million/ml and 20% men achieved azoospermia, and since that time all men had achieved sperm concentrations < 3 million/ml in the TU and DMPA group. In the 12 weeks, i.e. after second injections, the sperm concentrations became 13.24±2.17 million/ml, and 0% men achieved azoospermia in the TU group. The sperm concentrations became 0.02±0.01 million/ml, and 80% men achieved azoospermia in the TU and DMPA group. The remaining of 2 men (20%) achieved sperm concentration < 0.1 million/ml. It can be concluded that injections of TU and DMPA is a promising approach for effective contraception in Indonesian men.

P1/2 – 134

HUMAN SPERMATOZOAL MEMBRANE ASSOCIATED CHANGES IN PRESENCE OF RISUG-A NEW INJECTABLE MALE CONTRACEPTIVE
K. Chaudhury*, AK Bhattacharya*, and SK Guha*, Center for Biomedical Engineering, Indian Institute of Technology and All India Institute of Medical Sciences, New Delhi, INDIA. # Department of Biochemistry,Calcutta University, INDIA

Twenty five years of research has resulted in the development of a new reversible male contraceptive injected bilaterally into the lumen of the vas deferens. The contraceptive, given the name RISUG, is a polymer styrene maleic anhydride dissolved in dimethyl sulfoxide. Phase I and Phase II clinical trials confirmed the efficacy and non-toxicity of the drug. RISUG, when injected into the vas deferens, does not cause any sclerosing effects and because of its non-adhering properties can be flushed out of the lumen. To establish the functional competence of the sperm in presence of RISUG, the integrity of both plasma and acrosomal membrane systems has been evaluated. The study demonstrates that 5'-nucleotidase is released from the plasma membrane on treatment with RISUG for 5 min at 30 degrees C and 150% increase in the enzyme release has been observed indicating the destabilization of plasma membrane system. Under similar conditions, the cells when subjected to hypo-osmotic swelling test, demonstrate appearance of significantly less number of tail curling of sperms showing loss of osmolarity. Sperm proacrosin is also converted to free acrosin which gets degraded almost spontaneously in presence of RISUG. Out of total acrosin content (1275 ± 38 mlU/100 million sperm) that remains associated with the human sperms, almost 92% of the enzyme is released indicating the disruption of acrosome. The results show that RISUG, even at a very low concentration of 1 mg/ml, causes almost complete destabilization of both plasma and acrosomal membranes.

P1/2 – 135

EFFECTS OF A LONG-ACTING PROGESTAGEN IMPLANT (NORPLANT II) WITH ANDROGEN PREPARATIONS IN THE SUPPRESSION OF SPERMATOGENESIS IN NORMAL MEN.

IT Gau Gonzalez, M.D.*, RS Suerdloff, M.D., A Nelson, M.D., B Clevelenger*, R Garcia, M.D.*, N Berman, Ph.D. d* and C Wang, M.D. Department of Medicine and Pediatrics, and Obstetrics and Gynecology,< Harbor-UCLA Medical Center Research and Education Institute, Torrance, California, U.S.A.

Orally active progestagens such as levonorgestrel (LNG) or desogestrel when combined with weekly physiologic dose of testosterone enanthate (TE) (100 mg) injections suppress spermatogenesis more efficiently and rapidly than 100mg TE alone. We initially used combined Norplant II (NI) and T patches (TP) to test if steady state de-livery systems will be as effective. 39 healthy male volunteers, age 20 to 45, were assigned to: Group 1 (n=19), TP (10mg/day) alone; group 2 (n=20), combined NI (4 capsules = LNG 160 ug/day) +TP. Additional 26 subjects were randomized to: Group 3 (n=14), NI+TE injection (100 mg/week); group 4 (n=12), oral LNG (125g/day)+TP. Subjects received treatment for 24 weeks with a subsequent recovery period of 24 weeks. Azoospermia was achieved in 6%,22%,90% and 11% and severe oligozoospermia developed in 31%,55%,100% and 33% of the subjects in groups 1,2,3 and 4, respectively by week 21. NI+TE was significantly more efficient in suppressing spermatogenesis to azoospermia when compared with oral LNG+TP (p=0.001) and NI+TP (p =0.012). We conclude that NI1 + TE was very efficient in suppressing spermatogenesis to a level acceptable for contraceptive efficacy. This study also demonstrated the critical role of the dose and delivery of androgens in combined androgen-progestagen regimens for hormonal-based male contraception.

P1/2 – 136

ANTIFERTILITY EFFECT OF ACTIVE IMMUNIZATION WITH THE C-TERMINAL 67-94 REGION (R-28) OF HUMAN SEMINAL PLASMA INHIBIN

MN Mehta*, SD Mahale*, KSN Lyer*, GR Vanage* and SB Moodlehi*, Institute For Research In Reproduction, I.M. Street, Parel, Mumbai 400012, Mahanagar, India

Human seminal plasma inhibin (hSPl), also known as prostatic secretory protein (PSP-94), secreted by the prostatic epithelium has 90% structural homology with a sperm coating antigen, suggesting the possibility of using hSPl as a male contraceptive. Two segments -- one at the N-terminus (1-17) and the other at the C-terminus (67-94) -- were identified as potential antigenic peptides. The present study describes the results obtained with the C-terminal region of hSPl. The peptide was chemically synthesized by solid phase synthesis using Fmoc chemistry, purified by RP-HPLC and characterized by amino acid analysis and mass spectroscopy. The peptide was conjugated to diphtheria toxoid and adult male rabbits were immunized. Titres as high as 1:1,28,00 were obtained against the peptide. The antibodies recognized the native protein (hSPl) in the ELISA system, showed positive histochemical staining of the prostatic epithelium and agglutinated washed human, monkey and rat spermatozoa in vitro. Administration of the antibody to the adult male rats caused elevated FSH levels. When normal female rabbits were cohabitated with immunized males (n=10), 40% reduction in the litter size was observed. The serum testosterone levels of the immunized animals were unaffected and the inhibitory effect on the male fertility was reversible. Since the R-28 peptide contains two cystein residues, the peptide has been cyclized and used for immunization after conjugation with the toxoid. Initial results with the cyclic peptide have shown encouraging results. To conclude, our results suggest the possibility of using the C-terminal of hSPl for the development of a male contraceptive vaccine.
P1/2 – 137
SERUM GONADOTROPINS & GERM CELL DEVELOPMENT WITH TESTOSTERONE (T) + PROGESTIN CONTRACEPTION.
Contraceptive regimens using T +/- progestin act by suppressing FSH/LH levels & sperm counts but their relationships with the sites of spermatogenic impairment are unclear. T enanthate (200 mg im wky) +/- depot medroxyprogesterone acetate (DMPA. 300mg im once) were given to normal men for 2, 6 or 12 wks prior to vasectomy & testis biopsy for stereological estimation of germ cell populations. Serum & semen samples were taken weekly, serum FSH/LH were measured by modified Delfia assay methods with increased sensitivity.
The inclusion of DMPA led to a more rapid fall in serum FSH/LH levels (ED90: FSH; 8.5 vs 15 d: LH 2.7 vs 13 d, p<0.01, DMPA vs T respectively) yet the mean time to sperm count (ED90) was the same (32d vs 31d, NS). Maximum FSH/LH suppression was identical at 12 wks (mean FSH 1.6 & 1.2%, mean LH 0.2 & 0.3% of baseline) as was sperm count suppression (4/5 & 5/5 men with sperm counts <0.1x10⁹/ml, DMPA vs T). At 2 wks, type B spermatagonia (Sg) and early spermatocytes were lower in the DMPA group, however at 6 & 12 wks, germ cell numbers did not differ. Primary lesions were seen in Apale-IB Sg maturation and a striking inhibition of spermatiation, eg. at 6 wks in both groups, the number of elongated spermatids was NS compared to controls, yet sperm counts were <10% of baseline.
We conclude that: (i) the addition of DMPA hastens the onset of FSH/LH suppression, correlating with more marked impairment of Sg development, but in the longer term, neither germ cell no. nor sperm count differed, (ii) Sg inhibition is a consistent feature but spermatiation inhibition is also striking & appears the major determinant of sperm counts.

P1/2 – 138
CURCUMIN, A NATURAL HERB WITH ANTI-HIV ACTIVITY INHIBITS HUMAN SPERM MOTILITY. Mahadevan Rajasekaran, 'Tara Rithaporn*, Manoj Monga, UCSD Medical Center, San Diego, CA.
To evaluate the sperm immobilizing effects of curcumin, a plant derived diferuloylmethane compound.
Prospective studies to analyze time course of curcumin induced changes in human sperm motility and viability parameters and determine optimal dose of curcumin for sperm immobilization.
Washed human sperm obtained from fertile donors (n=3) were suspended (20-30 X 10⁶/ml) in HAM's F-10 and exposed to varying concentrations of curcumin (0-300µg/ml) for up to 2 hours at 37°C. Sperm motility was evaluated using a Makler chamber and viability was checked by Eosin-Y dye exclusion.
Incubation of normal human sperm with curcumin resulted in a dose and time dependent loss of sperm motility. At lower concentrations (30µg/mL), curcumin produced a 3-4-fold decrease in sperm motility with in 30 minutes without significant effects on sperm viability. An instantaneous (>90%) loss of sperm motility was observed with higher concentrations (300µg/mL) of curcumin.
Our results indicate that curcumin has a selective sperm immobilizing activity in addition to a previously studied anti-HIV property. This compound may have potential clinical applications as a novel intravaginal spermicidal agent for contraception and HIV prevention.

P1/2 – 139
EFFECT OF LEAF EXTRACT OF NEEM (AZADIRACHTA INDICA) ON THE REPRODUCTIVE ORGANS OF MALE MOUSE.
Raghav Kumar Mishra and Shio Kumar Singh, Department of Zoology, Banaras Hindu University, Varanasi, India.
The effect of oral administrations (100 mg and 200 mg/kg body weight/day, for 28 days) of leaf extract of neem on the male reproductive organs of Parkes (P) strain mice was investigated. Histologically, testes in mice treated with neem showed nonuniform regressive changes in the seminiferous tubules; both regressed and normal seminiferous tubules were observed in the same section. In general, the regressed seminiferous tubules showed intraepithelial vacuolation, exfoliation of germ cells, degeneration of germinal elements, and disorganization of the germinal epithelium. The frequency of regressed seminiferous tubules in the testis was dose- dependent. However, normal features were still observed in the majority of the seminiferous tubules in the testis of neem-treated mice. Neem treatment had also adverse effects on motility, viability, morphology, and number of spermatozoa in the cauda epididymis, and on sialic acid and fructose levels in the epididymis and seminal vesicle, respectively. Our results, thus, suggest that neem treatment in P mice causes marked alterations in the male reproductive organs.

P1/2 – 140
7α-METHYL-19-NORTESTOSTERONE (MENT™) IMPLANTS FOR MALE CONTRACEPTION: A DOSE-FINDING STUDY.
S. v. Eckardstein1, E. Nieschlag1, H. Croxatto2, G. Noé3, V. Brache4, F. Alvarez2, A. Moo-Young5 and K. Sundaram5, Institute of Reproductive Medicine1, Münster, Germany, Instituto de Medicina Reproductiva2, Santiago, Chile, PROFAMILIA3, Santo Domingo, Dominican Republic, Population Council4, New York, NY, USA MENT™, a synthetic androsten that is not convertible to dihydrotestosterone, is suitable for androgen substitution in male hypogonadism. In normal men intramuscular injections of MENT resulted in a profound suppression of gonadotropins. The present study investigated the suppressive effect of MENT on spermatogenesis in healthy men. MENT was delivered from either one, two or four implants each containing 135 mg of MENT acetate inserted subdermally and left in situ for 180 days. Thirty-four volunteers with normal semen values were recruited in 3 centers and randomly assigned to one of the doses. Semen analysis, serum LH, FSH, testosterone and MENT levels were monitored monthly. Safety parameters included clinical chemistry, hematology, PSA and standardized questionnaires on general well-being and sexual performance. In the 4-implant group, suppression to azosperma, or sperm counts of < 3 mill/ml occurred in 9/11 volunteers compared to 4/11 in the two implant-group and 0/12 in the single implant group. During treatment no serious general side effects, signs of androgen deficiency or extrusion of implants were observed. The results show that the effectiveness and safety of four MENT acetate implants are similar to those reported for testosterone enanthate injected weekly or testosterone undeconenate injected at 6-week intervals. Therefore, MENT offers an attractive modality for the development of a hormonal male contraceptive.
P1/2 – 141
FREQUENCY OF HERPES SIMPLEX VIRUS, CYTOMEGALOVIRUS AND HUMAN PAPILLOMAVIRUS DNA IN SEMEN.
O Aynaud, J-D Poveda. Hôpital Tarnier, 75006 Paris, Laboratoire Pasteur-Cerba

HSV2 and CMV infections produce brain damage in the newborn and human papillomavirus (HPV) plays a role in cervix carcinogenesis. The frequency of herpes virus and HPV in semen and its role in transmission are poorly known.

Semen from 111 male partners of women with histologically detected genital HPV infection was studied for HSV, CMV and HPV infection. We used cell culture to detect for HSV and CMV, and Southern blot and polymerase chain reaction for HPV. The presence of HSV DNA was correlated with detailed history and physical examination findings. As for HPV, virological findings in the sperm were correlated to the presence or absence of HPV-associated genital lesions ans the to viral type.

Viral cultures yielded HSV2-DNA in 9% and CMV-DNA in 6.3% of cases. No correlation was established with a history of clinically apparent infection for HSV. HPV-DNA was detected by PCR in 48% of subjects with uretal lesions, in 22% of semen samples from patients with penile lesions and no uretal lesions, but in only 2% without HPV-associated lesions. HPV-DNA type 16 was detected in three cases. Patient with positive sperm sample and penile or uretal lesions always presented the same HPV type in the two specimens.

The study shows a surprisingly high detection of clinically inaparent HSV and CMV but does not confirm high prevalence in semen from men without detectable lesions. Our study also suggests that the mechanism for sperm contamination by HPV is the exfoliation of infected cells from uretal lesions during sperm ejaculation and probably in patients with penile lesions resulting in the contamination of semen used in assisted reproductive technology.

P1/2 – 142
EFFECTIVENESS OF "SPERM WASHING" TO RECOVER SPERMATOZOA WITH-OUT HIV AND HCV GENOMES DETECTION IN HIV INFECTED MEN

Objectives: Many serodiscordant couples with HIV infected male partner are seeking medical assistance to reduce the risk of HIV transmission to have children. Before ART program, we conducted a study to demonstrate the efficiency of 'sperm washing' in reducing HIV-1 and HCV viral load. Material and methods: 94 patients performed 282 blood and semen samples. HIV-1 RNA levels were measured on blood and seminal plasma by a modified HIV-1 MonitorTM assay. A modified AmplicorTM assay was used to detect HCV in seminal plasma and semen cells. Isolation of motile spermatozoa was achieved by density gradient centrifugation (50%, 70%, 90% of Pure SpermTM) and then 90% fraction was submitted to a swim up method. After nuclear extraction HIV RNA and DNA were detected using a modified HIV-1 MonitorTM assay on whole sperm, 50 % fraction and on motile sperm obtained after swim-up.

Results: HIV RNA was detected in 142 blood and in 38 seminal plasma. In 106 cases the blood was positive and the seminal plasma negative, and in 8 cases the plasma seminal was positive while the blood was negative. 8.0 % of whole sperm cells and 5.3 % of 50 % fraction were positive. All tested motile spermatozoa swim up fraction were undetectable for HIV RNA and DNA (detection limit: 10 copies / 2 x 10^6 cells). HCV RNA detection was positive in 119 blood samples. 15 of 95 (15.8%) tested seminal plasma were RNA-HCV positive while none of the tested whole sperm cells (n=102). 50 % fraction (n=94) and motile sperm fraction (n=67) were RNA-HCV positive. Conclusions: Sperm processing used in this study was effective to reduce the HIV-1 RNA and DNA levels as motile sperm spermatozoa fraction had always HIV-1 RNA and DNA levels undetectable whatever the results of seminal plasma, whole sperm cells or 50% fraction cells. Moreover, using a standardized technique with internal control we demonstrated that HCV could be present in seminal plasma but we did not find HCV RNA in cells fractions.

P1/2 – 143
HIV SEROPOSITIVITY AND MALE INFERTILITY: A CLINICAL DILEMMA
Bormann MS, G Schuelenburg. Andrology, Departments of Urology, University of Pretoria and Medunsa, Pretoria, South Africa

Infertility is common in Africa, but essentially no data exist on HIV prevalence among infertile men. This study addresses the impact of the human immuno-deficiency virus (HIV) on clinical infertility practice. HIV causes the acquired immuno-deficiency syndrome (AIDS). This study was conducted in a hospital in northwest South Africa from June 1999 to January 2001. Men presenting with infertility problems to the outpatient clinic were interviewed, examined and blood was drawn after informed consent. HIV 1 and 2 antibody screen was performed on samples from 303 men. Of these, 70 samples (23.1%) were confirmed positive for HIV. The seropositive males and couples were counselled and no assisted reproduction techniques (ART) were considered. Diagnostic examinations and ART procedures should not carry the risk of transmitting any infection to the female or fetus (Diani 1999, Hargrave 1998). Women with fertility problems appear to have higher HIV prevalence (Favot 1997). Our findings justify that more attention should be directed to infertile couples in the context of AIDS programmes. Reference Diani F. Sexually-transmitted diseases and assisted reproduction techniques. Clinical & Experimental Obstetrics & Gynecology. 26(2):131-2, 1999. Hargrave TB. Ghosh C. The impact of HIV on a fertility problems clinic. Journal of Reproductive Immunology. 41(1-2):261-70, 1998 Dec. Favot I, Ngalula I, Mgalla Z, Klokke AH. Gumodoka B. Boerma JT. HIV infection and sexual behaviour among women with infertility in Tanzania: a hospital-based study. International Journal of Epidemiology. 26(2):414-9, 1997.

P1/2 – 144
Mycoplasma and Calamundy Esigien Factors of Conyugal Infertility
Lab. de Andrología, Depto. de Patología y Centro de Control de Enfermedades Infecciosas, Fac. de Medicina Universidad Autónoma de Nuevo León, México.*Departamento de Ciencias Morfológicas y Medicina Preventiva de la Facultad de Medicina, Universidad Autónoma de Madrid, España.

The Genital Infections (GI) are one of the aspects frequently underestimated like an infertility factor. We have studied subfertile patients with GI, the seven last year. They were associated to altered sperm concentration, motility, vitality and characteristic teratozoospermic pattern. In all of them we were present some types of bacteria adhered or included into epithelial, inflammatory and spermatic cells. The identified germs were S. faecalis (44%), F. coli (18%), S. aureus (38%) and S. epidendycts (32%), but the most frequently associated to seminal alterations and reproductive failure were Mycoplasma sp. (M. Sp): 66% and Chlamydia trachomatis (C. T): 38%. The initial diagnosis by specific media cultures of M. Sp., were improved with PCR for U. Urealyticum and Electron Microscopy studies. The presence of C. T., tested in a uretal smear by direct immunofluorescence (DIF) with monoclonal antibodies, were then confirmed by the same technique applied to semihin sections of the seminal bulb, examined by light microscope (LM). These bacterial particles were morphologically characterized and detected in the semen sections stained with toluidine blue. It was possible to found low bacterial concentrations undetected in the specific cultures or negatives for DIF. With these techniques, we diagnosed a high figure (89%) of infertility cases, that involves M. Sp y C. T in the seminal sample and were correlated with the finding of these germs in the female genital fluids as well as the GI symptoms, failure in assisted reproduction, recurrent and early miscarriage, anembrionic pregnancy, and intrarterial fetal death.

These data suggest that GI caused by M. Sp and C. T. are present in a high percentage of conjugal sterility and play a relevant etiological factor of this and other relevant reproductive problems. Many of failure (economic and medical) of assisted reproduction would be avoided with a diagnosis and treatment adequate of both members of couple with genitorium infections.
P1/2 - 145
NONSPECIFIC GENITAL INFECTIONS DIAGNOSED BY FRESH CYTOLOGICAL ANALYSIS.
Banca-González B. and G. Gallego-Avila. Lab. of Andrology, Pathology Dept. School of Medicine, Universidad Autonoma de Nuevo León, Mexico.

The non-specific genito-urinary infections (NSGI), are underlying in a high percentage of cases of couple with reproductive failure. The exact diagnosis and frequency are difficult to know, because many cases are subclinical and the cost, sensitivity and limited testing area, affect the interpretation and the general application of conventional microbiological probes.

We applied 1% central rod and 1% yellowish eosin stain, for the cytological examination of non-fixed seminal samples from infertile men and for vaginal smears of their wives. They had symptoms and seminal data of infectious disease. This microscopical analysis was done to detect morphologically the presence of Chlamydia (Ch) and Mycoplasma (My), the most frequent germ associated to NSGI. The results of this exam, were compared to Mycoplasma sp. specific antibodies (SCM) and direct immunofluorescence of anticlamydia monoclonal antibodies (DIF-Ch), carried out on the same samples. The obtained data, pointed out the presence of 0.2-0.3 μm pleomorphic bacteria, morphologically corresponding to mycoplasma in 74% of the studied samples, of them 89% were positive at the specific antigles. Any of the samples without the pleomorphic bacteria were positive to the SCM, but 11% of the cases with low concentration of pleomorphic bacteria, were negative at the culture. The direct bacterioscopic observations showed the presence of typical Chlamydial Inclusion Bodies in 46% of the cases, and 83 % of them were confirmed by DIF-Ch. 13% of men with DIF-Ch negative, but with Chlamydial Inclusion Bodies positives at fresh exam, had wife with DIF-Ch positive concomitant sample.

This results suggest that microscopical analysis, of non-fixed genital secretions, is useful to detect bacteria of etiological importance for NSGI. This fast, easy, and inexpensive method can be applied to high number of patients on the initial evaluation of infertile couple or other reproductive problems associated to NSGI.

P3/4 - 001
EXPRESION OF CFTR IN EJACULATED HUMAN SPERMATOZOA.

Cystic fibrosis transmembrane conductance regulator (CFTR) is a CAMP-activated chloride channel expressed in epithelial cells. CFTR mRNA has been previously identified in round spermatids and Sertoli cells by in situ hybridization. However, CFTR immunoreactivity has never been reported in spermatozoa. In this study, we report the expression of CFTR in ejaculated human spermatozoa. Semen samples were obtained from healthy donors and fractionated by Isolate density gradient centrifugation. Sperm in the 90% pellet were isolated and processed for immunocytochemical and Western blot analyses using monoclonal antibodies against human CFTR. Immunocytochemical analysis was carried out in frozen sperm pellets sectioned to 6μm thickness and affixed to glass slides. The slides were fixed in 80% acetone, treated with 0.25% Trypsin, blocked for endogenous avidin and biotin, followed by blocking with normal horse serum. The slides were exposed to anti-CFTR mAb13-1 (Genzyme Corporation, Framingham, MA) (1:100) overnight at 4°C, then exposed for 1h to biotinylated goat-anti mouse IgG (1:200), incubated for 1h with peroxidase conjugated streptavidin, and treated with 3,3 diaminobenzidine (mg/ml.) in fresh Tris buffered saline (TBS) and counterstained with methyl green. Sperm membrane reactive were extracted with 0.1% Triton X-100 in PBS, loaded onto 10% polyacrylamide gels, separated by SDS-PAGE electrophoresis, and transferred to Immobilon P membranes. The membranes were blocked with 0.5% BSA in PBS, incubated overnight with the mAb13-1 (1:500) in PBS containing 0.5% Tween-20 and 0.1% BSA, and then incubated with goat anti-mouse IgG-peroxidase. The resulting immunoreactive bands were developed using Amersham ECL chemiluminescent detection system. Immunocytochemical analysis revealed abundant expression of CFTR in the postcapitular, midpiece and principal piece regions of human spermatozoa. No immunoreactivity was detected in control spermatozoa in which the primary antibody had been omitted. The identity of CFTR was further characterized by Western blot analysis of sperm proteins revealing the presence of an immunoreactive band with an apparent molecular weight of 85-90kDa which corresponds to the molecular weight of monoclonic CFTR. No immunoreactive bands were observed when the primary antibody was omitted. Abundant expression of CFTR in mature spermatozoa suggests a role for CFTR in the regulation of sperm function. Studies are currently underway to measure CAMP-activated chloride conductance in human spermatozoa. This work was supported by a grant provided by the Lalar Foundation.

P1/2 - 146
EFFICIENCY OF ARTIFICIAL INSEMINATION IN GIANT PANDAS AT THE WOLONG BREEDING CENTER.
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The goal of the giant panda (Alluroopa melanoleuca) breeding program is to develop a self-sustaining, genetically diverse population. Due to the common problems of (1) sexual incompatibility and (2) a limited number of captive-bred males that breed naturally, artificial insemination (AI) has become a critical genetic management tool. The common practice, however, is to combine natural mating and AI using semen from non-breeding males. From 1998 through 2000 at the Wolong breeding center, 12 of 18 (66.7%) females produced 20 cubs following combined natural mating and AI. The objective of this study was to determine the efficiency of AI without natural breeding. In 1998 and 2000, a total of 10 females were inseminated over the period of AI on 53 days. Ejaculates from six males were collected by electroejaculation, diluted in an egg-yolk diluent containing 0 or 4% glycerol and used either fresh or following cold-storage at 4°C (for 24 or 48 h) or cryopreservation using the pellet freezing method. Mean (±SEM) ejaculate traits in six male sperm donors were: ejaculate volume, 3.3 ± 0.5 ml; sperm concentration, 1.429 ± 0.235 x 10⁶/ml; sperm motility, 81.7 ± 2.1%; progression (0.5-5), 3.1 ± 0.1; and normal sperm, 73.3 ± 9.2%. For AI (n = 14) in seven females, mean insemination traits were: sperm volume inseminated, 2.4 ± 0.3 ml; sperm motility, 73.5 ± 2.9%; progression, 2.5 ± 0.1; and total motile sperm inseminated/AI, 684 ± 118.2 x 10⁶. Four of seven (57.1%) females became pregnant and produced five cubs of which four survived. Mean gestation and litter size was 131.5 ± 9.7 days and 1.3 ± 0.3 cubs/litter, respectively. These results indicate that the efficiency of AI is sufficient for recovering valuable genes from non-breeding individuals to enhance genetic diversity in the ex situ population of giant pandas.

P3/4 - 002
SURFACE MAPPING OF OVUDCTIN OVER THE PLASMA MEMBRANE OF GOLDEN HAMSTER SPERMATOZOA DURING IN VITRO CAPACITATION AND ACROSOME REACTION.
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We examined the binding of oviductin to the plasma membrane domains of non-capacitated, capacitated, and acrosome-reacted hamster spermatozoa using indirect immunofluorescence, immunoelectron microscopy and surface replica techniques. Freshly prepared non-capacitated, capacitated and acrosome-reacted spermatozoa were incubated in oviducal fluid, fixed and then labeled with a monoclonal antibody against hamster oviductin. Indirect immunofluorescence revealed binding of oviductin to the acrosomal cap and the postacrosomal region of both non-capacitated and capacitated sperm. Acrosome-reacted sperm displayed an moderate immunostaining pattern in the postacrosomal region. In all three experimental conditions, thin-sections of Epon-embedded sperm previously labeled with antibody-protein A-gold complex showed gold labeling confined to the aforementioned plasma membrane domains. Surface replica preparations provided the en-face view of surface mapping of oviductin in the acrosomal cap, postacrosomal region, and equatorial segment of non-capacitated and capacitated sperm and in the postacrosomal region and equatorial segment of acrosome-reacted samples. Results obtained from quantitative analysis indicate that oviductin has a higher binding affinity for the acrosomal cap than the postacrosomal region and that binding of oviductin to the latter domain is enhanced during capacitation but attenuated after acrosome reaction. Hamster oviductin appears to have the lowest affinity of binding for the equatorial segment regardless of the experimental conditions. The binding of oviductin to sperm and its redistribution during capacitation and acrosome reaction are likely to influence the sperm-egg interaction.
P3/4 – 003

COMPOSITION AND DEVELOPMENTAL EXPRESSION OF PROTEINS CONSTITUTING THE SPERM HEAD CYTOSKELETON OF THE PLAINS RAT.

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The Plains rat, Pseudomys australis, has a distinctive falciform sperm head structure containing an apical hook and two large ventral processes (VPs). These VPs have been isolated and shown to be composed of periforratorial cross-reacting proteins and F-actin. Antibodies raised against the perinuclear theca (PT) proteins, exemplary of spatiatile sperm head shape, were found to be restricted to regions of the apical hook and VPs adjacent to the acrosomal and nuclear membranes. The first objective of this study was to analyze the protein composition of the alkaline extracted Plains rat periforratorial-perinuclear theca (PERF-PT) complex on western blots, using antibodies raised against the laboratory rat PERF and the bull PT as probes. Our second objective was to evaluate and compare the expression and assembly pattern of PERF versus PT proteins during spermiogenesis in the Plains rat. Immunoprobng of western blot transferred PERF-PT extract revealed that most of the prominent PERF and PT proteins found in the laboratory rat and bull sperm, respectively, were present in the Plains rat. The predominant polypeptide of the extracted proteins was the 15 kDa protein (PERF 15) and in relatively lesser quantities a 34 kDa protein (PERF 34) and PT proteins PT 15 (SubH28v), PT 32 and PT 60 (calcin). Immunocytochemistry on testicular sections of the Plains rat testis showed that PT proteins were intimately associated, both temporally and spatially, with acrosome formation from the beginning of spermiogenesis, while PERF proteins began to assemble later in the subsacrosomal region of the PT in the elongating spermatids. (NSERC and ARC)

P3/4 – 004

ISOLATION AND PERINUCLEAR IMMUNOLOCALIZATION OF SOMATIC HISTONES H2B AND H3 IN BULL SPERM HEADS.

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During spermatogenesis, nuclear condensation is marked by the replacement of somatic and testis-specific histones by highly basic transition proteins and protamines. Though relative amounts of histones reported within sperm nuclei vary between species, we provide evidence that nucleoproteins may not be restricted to the nucleus. Isolated bull sperm heads whose nuclear envelope appeared intact were incubated in 1M KCl. The extracted proteins were separated by SDS-PAGE, revealing three major protein bands of 14-, 16-, and 17-kDa. Amino-terminal sequence analysis showed that the 14 kDa band was identical to a downstream sequence of somatic histone H3. The 16- and 17-kDa bands exhibited sequences identical to somatic histone H2B. Polyclonal antisera raised to the extract were affinity purified (AP) against the 14-, 16-, and 17-kDa bands. Western blotting revealed the AP antisera and anti-H2B antibodies cross-reacted with all three bands, whereas anti-H3 antibodies labeled only the 14 kDa band. Acid extracted proteins from fresh bull sperm were probed by anti-H2B and H3 antibodies, and single bands were respectively labeled, suggesting 1M KCI extracts contained breakdown of full histone sequences. The antisera generated from the 1M KCI extract also labeled all core somatic histone subtypes isolated from calf thymus. EM immunogold labeling using the AP antisera was exclusive to the perinuclear theca of spermatooza and late spermatids, with no significant nuclear labeling. This unprecedented perinuclear localization of histones in mature sperm suggests involvement in male pronuclear formation during fertilization. (Supported by the CIHR and NSERC)

P3/4 – 005

EXPRESSION OF P2Y PURINERGIC RECEPTOR IN HUMAN SPERM

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Introduction. Extracellular ATP triggers different responses in several cell types interacting with plasma membrane (PM) receptors named P2 purinergic (P2R) that are divided in two families: P2XR and P2YR. P2XR are ligand-gated ion channels activated by ATP while P2YR are coupled to diacylglycerol/IP3 formation and are stimulated by ATP and UTP. In this study we investigated the presence of P2YR in human sperm. Materials and Methods. Motile sperm from 5 fertile donors were utilized for the experiments. [Ca2+]i was evaluated using fura-2 and PM potential was monitored using bis-oxonol. Acrosome reaction (AR) was evaluated after two hours incubation with UTP. Total RNA was extracted from sperm and subjected to RT-PCR. Different sets of primers based on the published human P2YR sequence were designed for PCR experiments. Results and Discussion. PCR experiments revealed the expression of the P2Y2R subtype in human sperm. UTP induced a peak in [Ca2+]i followed by a plateau that was absent in Ca2+-free medium. PM potential monitoring showed that UTP induced a hyperpolarization inhibited by incubation with K+ channels blocker TEA. PM hyperpolarization was present also in Ca2+-free medium but was transient and is probably due to the opening of Ca2+-dependent K+ channels. UTP stimulated the AR but only in Ca2+ medium and incubation with TEA importantly reduced the effects of UTP on AR. These data show that human sperm express functional P2Y2R coupled to stimulation of [Ca2+]i rise and PM hyperpolarization that lead to AR.

P3/4 – 006

ISOLATION AND CHARACTERIZATION OF CAPACITATING FACTOR (GLYCOSAMINOGLYCANS) FROM THE BOVINE FOLLICULAR FLUID.


The role of glycosaminoglycans (GAGs) is one of the most studied pathways in the mechanism of the bovine sperm capacitation. For several years, we have been working on a group of proteins present in seminal plasma (the BSP proteins) which potentiate capacitation induced by heparin. However, heparin is not present in the female genital tract fluids. In this study, we investigated the role of lipoproteins depleted follicular fluid (LD-FF fraction) which contained all of the constituents of FF except the high-density lipoprotein particles in sperm capacitation. Our results indicate that the LD-FF fraction stimulates capacitation only when the BSP proteins were present on the sperm membrane. These results are very similar to the phenomenon observed with heparin. The effect observed with LD-FF fraction could be due to the presence of heparin-like GAGs in the bovine FF. Therefore, in the second step, we attempted to isolate large amount of GAGs from FF and characterize them. FF-GAGs were isolated from FF by protease digestion, lipid extraction and followed by different precipitation conditions. The different GAGs were purified by ion exchange chromatography on DEAE-Sephadex using different gradient of salt to optimize the separation. Then, isolated GAGs were characterized by agarose gel electrophoresis. Two GAGs, heparan sulfate and chondroitin sulfates, B, were present in bovine FF. In the near future, we will determine if these GAGs interact with the BSP proteins and induce capacitation in the presence of the BSP proteins.
P3/4 - 007
A COMPARATIVE STUDY OF THREE BUFFER SYSTEMS OF YOLK-FREE HUMAN SEMEN CRYOPROTECTIVE MEDIA. Yao Kang-Shou1, and Wei-Jie Zhu2, 1Zhe-Jiang Provincial Institute of Planned Parenthood Research, Hangzhou 310012; 2Center for Reproductive Immunology Research, Jinan University, Guangzhou 510632, China

Cryoprotective medium (CM) plays an essential role in the cryopreservation of human semen. Several studies and our previous work have demonstrated that yolk-free CM is effective for cryopreserving human sperm. However, there is a need to evaluate the effect of different buffer systems of yolk-free CM on sperm cryosurvival. The purpose of this study was to investigate the efficiency of three buffer systems of yolk-free CM. Three buffer systems: Tyrodine, BWW, and Ham's F10 physiological media, were used as the basic media of yolk-free CM, which were added into glycerol (10%-15%, v/v), sucrose (25-50 mM), glycine (I 33 mM), and human serum albumin (5 g/mL). A routine freezing method was employed to cryopreserve human semen. The results showed that after cryopreservation there was no significant difference in sperm motility, and sperm head to tail membrane integrity rate among these three CM (n=20, P>0.05). Sperm zona-free hamster oocyte penetration rate (n=10), and motility longevity (n=5) of these three yolk-free CM were observed in a similar range. Post-thawing motility of Ham's F10 CM containing 10% or 15% glycerol, and 25 mM or 50 mM sucrose showed no significant difference (n=10, P>0.05). From these results, it indicated that the experiment three buffer systems of yolk-free CM would have the similar efficiency for the cryopreservation of human semen. Reducing the concentration of glycerol and sucrose of yolk-free CM would not significantly influence sperm cryosurvival. (Supported by Guangdong NSF)

P3/4 - 008
ENHANCED CAT SPERM CRYOPRESERVATION BS Pukazhenth, DE Wild*, and JG Howard, Conservation & Research Center, National Zoological Park, Smithsonian Institution, Front Royal, VA.

To study the ability of cat sperm to survive cryopreservation. we examined the effect of: 1) cryomethod (pellet versus straw), 2) slow cooling and addition of glycerol at 4°C, 3) egg yolk concentration and multi-step removal of glycerol, and 4) methyl-β-cyclodextrin (MBCD) in normospermic (N; >60% normal sperm/jaculate) and teratospermic (T; <40% normal sperm) domestic cats. Electroejaculates (3-5 males/group, 1 ejaculate/male) were diluted in Ham's F10 + HEPES (HF10), washed and pellets reconstituted in TEST yolk buffer (TYB). In Study 1, sperm pellets were reconstituted in TYB + 20% egg yolk + 4% glycerol at room temperature; slow cooled to 4°C (2 h), and cryopreserved in straws (on dry ice) or 0.25 ml straws (over liquid nitrogen vapor). In Study 2, sperm pellets were reconstituted in TYB + 20% or 5% egg yolk supplemented with 0, 10 and 20 mM MBCD, cooled to 4°C and cryopreserved in 4% glycerol in straws. Pellets were thawed (10 s in air, 30 s in 150 μl HF10, 37°C) in a glass tube. Straws were held in air for 10 s and immersed in a water bath (37°C) for 30 s. Thawed semen was diluted in HF10 slowly (60 s) (Study 1) or in 8 fixed volume steps at 20 s intervals (Study 2). Percent sperm motility and intact acrosomes (IA) (Coomassie stain) were assessed at 0, 3 and 6 h. Pellet and straw freezing resulted in similar (P>0.05) sperm motility and <30% IA in both cat populations. Egg yolk concentration had no effect. In contrast, when cooled slowly and glycerolated at 4°C, sperm from N males retained more (P<0.01) IA (70 ± 1.5%) compared to T cats (31 ± 1.0%). Removing glycerol in 8 steps resulted in a slight (P>0.05) decline in IA in N (56 ± 2.1%) and T (23 ± 3.7%) populations. MBCD exposure had no positive effect and was toxic to sperm from N males (at 20 mM). Thus, combined slow cooling, glycerol addition at 4°C and multi-step glycerol removal improves sperm acrosomal integrity in N male cats. Enhancing sperm survival in T males will require additional studies. (NIH KO1RR00135 and Smithsonian Institution's Scholarly Studies Program).

P3/4 - 009
THE EFFECT OF PROSTAGLANDINS ON CHILDBIRTHING FUNCTION OF WOMEN INSEMINATED WITH FROZEN-THAWED SPERM. Grigoryan SB*, Avagyan VV*, Nazaryan VK* *Huys-M* Department of Primary Health Care Centre of Armenia, Moskvan str. 15, Yerevan-375001, Armenia

The effects of prostaglandins as a new class of bioactive substance's in the treatment of different diseases and particularly in stimulation of reproductive function of women is widely known. It was shown that prostaglandins are contained in all tissues, but in the ejaculate its content is about a hundred times higher. Studies of M. Bygedeman (1968) and Giltsova NZ, Golub VS. and others (1974) have proven that the prostaglandins have a stimulating effect on the cervical dilation in the process of insemination of sheeps in the period of ovulation and promotes the movement and approach of spermatozoon to the ovocyte. The studies of Milovanova and Sokolevskaya (1980) confirm that in the process of cryoconservation 79% of prostaglandins are destroyed. The aim of the present study is to check the efficacy of adding of prostaglandin F2α (PGF2α) to the frozen thawed human semen used for artificial insemination of women. The experimental data show, that addition of prostaglandin F2α to the frozen-thawed human semen leads to a reliable increase of efficacy of women's insemination by 20%.

P3/4 - 010
THE MEDIUM FOR LOW-TEMPERATURE CONSERVATION OF HUMAN SPERM. Grigoryan SB*, Avagyan VV*, Nazaryan VK* *Huys-M* Department of Primary Health Care Centre of Armenia, Moskovan str. 15, Yerevan-375001, Armenia

In recent years there is a tendency towards an increase in the number of infertile married couples due to male infertility. In this case the treatment is realized using artificial insemination with donor's sperm. There exist various media for low-temperature conservation of human sperm that includes different expensive components (glucose, glycine, hen's egg yolk, glycercin, etc.). According to the literature data, the efficiency of insemination with frozen-thawed sperm is 44-64%. The aim of the present study was to improve spermability after cryoconservation. In the process of technological treatment and preparation of the sperm for cryoconservation there occur overoxidation of the sperm acrosomes, amino acids, inactivation of prostaglandin's and the resultant decrease in spermatozability. The novelty of the study is that we, for the first time, added to our developed medium the antioxidant (monoothanolamine) and calcium sodium salt solution of EDTA (ethylenediaminetetraacetic acid) which inhibited oxidation of lipids an amino acids in spermatozoons. Our innovation allowed us to enhance the efficiency of the proposed medium and to provide sperm fertility up to 60-65%.
P3/4 – 011
INFLUENCE OF BOVINE OVUDICT EPITHELIAL CELL APICAL PLASMA MEMBRANE ON CRYOPRESERVED SPERMATOZOA.
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In bovine species, sperms are stored in the oviduct before fertilization by binding to oviduct epithelial cell apical plasma membrane. As the oviduct is able to maintain motility and viability of sperm and modulate capacitation, we propose that apical plasma membrane of oviduct epithelial cells contributes to this effect. To verify this hypothesis, frozen-thawed sperm were incubated for 6h with purified apical plasma membranes from fresh oviduct epithelial cells, from cultured oviduct epithelial cells and from bovine mammary gland cells as a control and motility percentage was determined. Fluorescence assisted cell sorting was performed on sperm incubated with fresh membranes using Indo 1 am to assess the membrane effect on intracellular calcium concentration of sperm. Fresh and cultured apical membranes succeeded to maintain initial motility (64.5% and 84.3% respectively) better than the control (without oviduct epithelial cell membranes) or mammary gland cell membranes. Apical membranes from oviduct epithelial cells diminished the number of sperm that reached a lethal calcium concentration on a 4h period (18%) compared to the control (54.6%) and maintained lower intracellular calcium levels in viable spermatozoa. These results show that apical plasma membrane of bovine oviduct epithelial cells surely contains factors that contribute to maintain motility and viability and to modulate capacitation of bovine sperm.

P3/4 – 012
LONG-TERM OUTCOMES OF ELECTIVE SPERM CRYOSTORAGE
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Long-term outcomes of elective sperm cryostorage programs are little reported. Over 22 years, 930 men sought elective sperm cryostorage in a single academic hospital. Of these men, 833 (90%) were able to have sperm cryostored while 13 (1%) were too ill to provide semen and 84 (9%) had samples unsuitable for cryo-storage due to azoospermia (31, 3%) or zero post-thaw motility (53, 6%). Sperm concentration was similar for testicular tumors and lymphomas but significantly higher among men with other cancers and non-malignant diseases. Among those whose sperm was cryo-stored, 141 (15%) had died and of these, 120 (85% of those dying) had their sperm discarded as contracted cryo-storage. Despite written agreement to discard sperm at death, requests to prolong cryo-storage after death were received from relatives of 21 men (2% of all applicants, 15% of deceased). Among 692 (74%) surviving their illness, sperm was discarded for 193 men (21% of all applicants, 28% of survivors) and cryo-stored sperm was used for 64 men (7% of all applicants, 9% of survivors) in 85 treatment cycles. Pregnancy was most efficiently produced by ICSI (median 4 cycles) compared with conventional IVF or artificial insemination. Most of the 21 requests for continued cryo-storage post-mortem arose in the immediate bereavement period (median 1 week after man’s death); only 3 cases had sperm transferred for use with no pregnancies reported. No demographic, fertility or seminal variables predicted the likelihood of requesting sperm usage or post-mortem continuation. In summary, the usage of cryo-stored sperm is sparse, but most effective using ICSI. Sperm cryopreservation allows virtually all men with threatened fertility to preserve their progressive potential.

P3/4 – 013
EFFECTS OF BICARBONATE ON HEAD-TO-HEAD AGGLUTINATION IN BOAR SPERMATOZOA.
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When boar spermatozoa are incubated in a medium designed for IVF, many of them are agglutinated with one another at the acrosome. Our previous data indicated that release of an epididymal protein "Anti-Agglutination" (AA) from the sperm acrosomes is required for the head-to-head agglutination. Moreover, capacitation-supporting factors including calcium and serum albumin play important roles in the control of this event. To reveal a mechanism of sperm agglutination, we examined the effects of another capacitation-supporting factor "bicarbonate" on sperm agglutination. Spermatozoa were collected from six mature boars, washed and resuspended in a modified Krebs-Ringer Heps lacking calcium chloride that was supplemented with sodium bicarbonate. The sperm suspension was incubated in a water bath (38.5 °C) for 1 h, and then used to determine the percentages of head-to-head agglutinated spermatozoa and to detect sperm-bound AA by SDS-PAGE and Western blotting techniques. The supplementation of sodium bicarbonate (5-10 mM) significantly raised the percentages of head-to-head agglutinated spermatozoa in the samples incubated in the presence of 25 µM phosphodiesterase inhibitors (IBMX, papaverine, and rosinaram) or 1 mM cAMP analogue (dbcAMP). However, the effects of sodium bicarbonate on sperm agglutination were reduced by a treatment with an inhibitor of the anion transporter (500 µM DIDS). Moreover, the relative amount of sperm-bound AA was significantly less in the samples incubated with 5 mM sodium bicarbonate in the presence of 25 µM IBMX, compared with the control samples. These data suggest that head-to-head agglutination in boar spermatozoa is controlled via a signaling cascade that is mediated by bicarbonate and cAMP.

P3/4 – 014
COMET ASSAY RELIABILITY IN CRYOPRESERVED HUMAN SPERM CELLS.

To identify the most reliable cryopreservation method in human sperm, semen samples from 16 men presenting for infertility evaluation at Massachusetts General Hospital were cryopreserved using four methods: flash freezing with and without cryopreservation, and programmed freezing with and without cryopreservation. To flash freeze we immersed the semen into liquid nitrogen (LN). Programmable freezing lowered semen temperature 1°C/min from room temp to 4°C followed by a 5 minute pause then a decrease of 8°C/min to -80°C followed by immersion in LN. Cryopreservative used was Medi-Cult brand sperm freezing media in 1:1 dilution with semen allowing 10 minute equilibration period prior to freezing. The straws were thawed in a 37°C water bath for 10 seconds. Neutral microei electrophoresis was performed and comets were stained with YOYO-1. Total comet length from 200 sperm was measured using an eyepiece micrometer at 400X magnification.

Pairwise intraclass correlations were computed to compare the fresh semen sample (gold standard) to each of the four freezing methods using a mixed effects linear regression model applied to each individual’s mean comet length for each freezing method. We found high correlations with the gold standard for the two freezing methods (flash and slow) in the absence of cryopreservation, R=0.69 and 0.71, respectively, but lower correlations in the presence of cryopreservation, R=0.43 and 0.45, respectively. In conclusion, using fresh semen samples as the ‘gold standard’, we showed that flash freezing or slow freezing without cryopreservation most closely reproduced the results obtained from using fresh human semen samples.
Abstracts – Poster Session 3/4

P3/4 – 015

THE ROLE OF A FUCOSE-BINDING PROTEIN IN SPERM BINDING TO BOVINE OVIDUCTAL EPITHELIUM.

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Sperm deposited in the female reproductive tract of some mammals are trapped in the isthmus of the oviduct by binding to epithelial cells, thereby forming a sperm reservoir. In cattle, sperm bind to a fucosylated ligand on the oviductal epithelium. We isolated a fucose-binding protein from sperm using affinity chromatography and examined whether it would inhibit binding of sperm to explants of oviductal epithelium. Washed ejaculated sperm were added to explants in the presence of fucose-binding protein, protein that did not bind to the affinity column (pass-thru), or buffer. After 15 min, loosely bound sperm were removed from explants by pipetting, and explants were videotaped to determine the density of bound sperm. Sperm binding density was significantly lower with fucose-binding protein (mean ± SE: 3.5±1.5 sperm/(0.1mm²)² explant) than with pass-thru proteins (14.7±2.4) or buffer (17.6±1.1). The fucose-binding protein was identified by amino acid sequencing as PDC-109, a seminal plasma protein. Therefore, PDC-109 was purified from seminal plasma and its effects on binding density of capacitated sperm were examined. Since capacitated sperm lose binding affinity for epithelium, binding density of capacitated sperm (8±2.5) was significantly lower than that of uncapacitated sperm (16±2), unless treated with PDC-109 (20±4). These results indicate that PDC-109 is involved in mediating binding of bovine sperm to oviductal epithelium during formation of the sperm reservoir.

P3/4 – 016

REGULATION OF HUMAN SPERM VOLUME

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Recently we have suggested defects in sperm volume regulation as the cause of infertility in the c-ras knockout mouse (Biol Reprod 61:1062, 1999; 63:612, 2000). The present work demonstrates the significance of this process on human sperm function. Swelling of washed ejaculated sperm was detectable by flow cytometry and light microscopy after treatment with quinine (20 - 300 μM), an ion-channel blocker known to inhibit regulatory volume decrease. Although percentage motility was unaffected, curvilinear velocity was increased with decreased straight-line velocity in a dose-dependent manner. Such kinematic effects were also inducible in semen and in artificial cervical mucus. In the presence of 0.5 μM quinine, penetration of sperm from semen into the mucus and migration through it was markedly reduced.

The mechanism of the quinine action on sperm volume and kinematics was examined using various ion channel blockers and ionophores. The effects of 125 μM quinine on washed sperm persisted to some extents in Ca²⁺-free medium, but were abolished by 1 and 5 μM valinomycin and 0.5 and 1 μM gramicidin. The results suggest that volume regulation in human sperm and the linear trajectory of their motion rely on some quinine-sensitive and TEA (tetra-ethylammonium chloride)-insensitive, largely Ca²⁺-independent K⁺-channels and possibly volume-sensitive organic anion channels.

P3/4 – 017

EFFECTS OF EXTRACELLULAR pH AND BUFFERING AGENTS ON MOTILITY OF FROZEN/THAWED BOVINE SPERMATOZOA.

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The control of extra and intracellular pH (pHe and pHf) can influence both the survival and capacitation of spermatozoa. Upon thawing, a large proportion of spermatozoa die or become moribund, which represent an expensive loss for the artificial insemination (AI) industry. A faulty control of pHf during freezing/thawing may be implicated in this phenomenon. Actually, the bovine AI industry often uses TRIS as buffering agent, which might be a problem due to its pHa (8.3) higher than the optimal pHf or pHf and its buffering capacity which is greatly affected by temperature. In this study, we used buffers weakly affected by temperature and with pHa closer to sperm physiology: BES (pHa 7.2), MOPS (pHa 7.2),PIPES (pHa 6.8), and HEPES (pHa 7.6). The capacity of these buffers to support sperm motility was evaluated at 10, 25 and 50μM in T-therm medium without HEPES and NaHCO₃ using 10x10⁶ spermatozoa/ml. These buffers were compared with TALP. Spermatozoa's motility was measured with computer assisted semen analysis after 2 and 6 hours incubation. This experience shows that only BES 10 and 25μM is better than TALP. After 6 hours incubation, 20% and 30% of motility was observed in BES 10 and 25μM respectively, compared to 5% in TALP. The resulting pHf of these solutions was 6.6 for BES 10 or 25μM and 7.2 for TALP, although an initial pHf of 6.8. In TALP medium, NaHCO₃ elevated between 0.4 to 0.6 pHf units: TALP+NaHCO₃ with 7.1 to 7.3 and TALP+NaHCO₃ with 6.6 to 6.7 respectively, after 2 and 6 hours. On the other hand, 0% motility was observed when the sperm were incubated for 2 hours in PIPES 50μM. These results will be helpful in studying how pHf control's affect pHf bovine spermatozoa upon cryopreservation. Supported by NSERC.

P3/4 – 018

THE HYPO-Osmotic SWELLING (HOS) CLASSIFICATION AND GRADING SYSTEM OF HUMAN SPERM.

FN Wang* and BY Suh, Dept of Ob/Gyn, King/Drew Med Ctr, Charles R Drew Univ L.A.,CA

Functional integrity of membrane is crucial for sperm(SPM) capactitation and acrosome reaction. Past study described shapes of HOS SPM but lack of a morphological classification and grading system to demonstrate clinical significance. The HOS Classification and Grading System was first proposed in this study:(a) swollen tail curled and end of tail touching to low end margin of SPM head, neck, or upper third of length from neck to tail tip was referred to Swelling Grade 3(SG3);(b) touching to middle third of tail was classified as SG2(c) nearby lower third of tail, or a shortened, overlapped and thickened tail, or any other forms of minimal swelling was listed as SG1:(d) non-swollen coiled tail with clarity in its coiled circle occurring at any piece from neck to tip was defined as SGO-R(non swollen but reactive);(e) completely non-swollen, non-reactive tail was regarded as SG0. Thirty fresh semen(SF) were split into 4 groups each and processed by Washing(W), Swim Up(SU), 2-layer Column(LC) and Real Time Micro-separation System(RT) according to protocols. Distributions of % morphology grading for HOS(Mean±SE), including SG3, SG2, SG1, SGO-R and SGO before and after treatment were analyzed. The common pattern shown in succession was SGO>SG1>SG3>SG2>SG0-R in FS, while SG3 became the most prevalent swollen form in preparations. RT collected the highest % of SG3 swollen SPM(53.7±3.9%), quadruple of SG2, as compared to SU(26.3±4.6%), LC (20.6±4.4%), W(23.9±3.9%) and FS(17.7±2.8%). (P<0.05,each). SPM with SGO-R shown in FS and preparation of W, SU or RT was few(2.5% or less). By contrast, LC yielded more number of SGO-R(5.3±1.2%) or SGO SPM(46.6±5.6%) as compared to RT and SU(P<0.05, V,each). We suggest The HOS Classification and Grading System is an indicator to further assess sperm quality.
P3/4 – 019

ANALYSIS OF ASIAN mtDNA HAPLOGROUPS IN LOW AND NORMAL SPERM MOTILITY

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Mitochondrial dysfunction reduces energy production and results in symptoms from various cells and tissues, including sperm dysfunction. A variety of mtDNA mutations responsible for human diseases have been related with low sperm motility. In this study, we have examined variants of mtDNA mutations in Asia or known as Asian mtDNA haplogroups in sperm of Indonesian men. Sperm samples from 57 donors were collected and analysed by standard procedures. Then, total DNA extraction and mtDNA PCR amplification, followed by restriction enzyme (HaeIII, Hpal, HincII, Ddel, Alul and deletion of mtDNA 9 bp) analysis were performed. Each donor was identified to one of the different haplogroups (A, B, F, M, M-C, M-D) as previously described. To investigate the potential association between mtDNA variants and sperm motility, we divided our sample in two groups: 16 low motility (asthenozoospermia) and 41 normal motility (normozoospermia) semen samples, and we estimated the frequency of each haplogroup in both populations. Haplogroups A, B and M could be detected in asthenozoospermia samples, of which haplogroup B was the most frequent (68.8%). In normozoospermia samples, haplogroups M, B, F and M-D could be detected, of which haplogroup M was the most frequent (46.3%). This study shows that particular variants of mtDNA mutations in Asia are significantly more abundant in asthenozoospermia samples or in normozoospermia samples, and this may be a relevant contributing factor to understand the cause of sperm dysfunction.

P3/4 – 020

PLATELET-ACTIVATING FACTOR ACTIVITY LEVELS (LIGAND AND RECEPTOR TRANSCRIPT) CONTENT IN SPERMATOZOA: MOTILE VERSUS NONMOTILE.

ET Purnell* and WE Rouleubush. Reproductive Biology Associates, Atlanta, GA, USA.

Introduction: Platelet-activating factor [1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine; PAF] is a unique signaling phospholipid which has pleiotropic biological properties in addition to platelet activation. PAF-ligand and PAF-receptor mRNA are both present in sperm. Platelet-activating factor plays a significant role in sperm motility, fertilization and subsequent preimplantation embryo development. Objective: To determine the activity level of PAF (ligand and receptor transcript) content in motile and nonmotile sperm. Design: Comparison of PAF activity levels (ligand and receptor mRNA) between motile and nonmotile sperm. Methods: Sperm samples were separated by density centrifugation into motile and nonmotile populations. PAF-ligand levels in sperm were measured via a PAF specific RIA. PAF-receptor mRNA expression levels in sperm were determined by semi-quantitative RT-PCR. Results: The PAF-ligand was detected in all sperm samples. The amount of PAF-ligand in the nonmotile sperm population (0.94 pm/million cells) was significantly higher (P<0.01) than the amount of PAF-ligand in the motile sperm population (0.29 pm/million cells). PAF-receptor mRNA was detected in all sperm samples. The amount of PAF-receptor mRNA in the motile sperm population (15.5 am/million cells) was significantly higher (P<0.01) than the nonmotile sperm population (6.8 am/million cells). Conclusion: There is an inverse relationship between PAF-ligand content in sperm, nonmotile sperm have more PAF than motile sperm. However, nonmotile sperm have a significant reduction of PAF-receptor mRNA. Nonmotile sperm may have more PAF-ligand due to their inability (lack of PAF-receptors) to utilize the compound.

P3/4 – 021

TREATING BULL SPERM WITH CHOLESTEROL LOADED CYCLODEXTRIN IMPROVES CRYO SURVIVAL.

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The objectives of this study were to determine if adding cholesterol loaded cyclodextrins (CLC) to bull sperm increased the percentages of motile cells following cryopreservation and to optimize a cryopreservation protocol using CLC in two cryopreservation media, Tris (T) and sodium citrate (NaC). Semen was collected from 9 Holstein bulls, diluted to 120 x 10^6 cells in 1ml of T or NaC diluent and then 0, 0.75, 1.5, 3.0, 4.5, 6.0 and 7.5mg CLC were added and cells incubated at 25°C for 15min (only 0 and 1.5mg CLC were added to NaC diluent). The samples were then diluted 1:1 with respective diluents containing 40% egg yolk (final egg yolk concentration of 20%) and then cooled to 5°C. Finally, a third fraction of respective diluents containing 20% egg yolk and either 14% glycerol (T diluent) or 16% glycerol (NaC diluent) was added. Afteraddition of glycerol, sperm were packaged into 0.5ml straws, frozen in liquid nitrogen vapor and stored in liquid nitrogen. Straws were thawed in a 37°C water bath and each sample evaluated using a Hamilton Thorne Motility Analyzer. Treatment differences in the percentages of motile cells were determined using a paired T-test. The percentages of motile sperm treated with 0 (control), 0.75, 1.5, 3.0, 4.5, 6.0 and 7.5mg CLC in T diluent were 42, 58, 60*, 57, 54, 53 and 48%, respectively; and were 39 and 50% for sperm treated with 0 or 1.5mg CLC in the NaC diluent. Bull sperm frozen in T diluent containing 1.5mg CLC resulted in higher post thaw motility (60%) than sperm frozen in T alone (42%; P<0.05). These results indicate that addition of CLC results in higher percentages of motile bull spermatozoa after cryopreservation and that 1.5mg CLC results in optimal post thaw recovery. Supported by USDA 2000-02410 and the NAAB.

P3/4 – 022

LOCALIZATION OF SP22 ON HUMAN SPERM OF DIFFERING QUALITY

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SP22 is a sperm membrane protein that has been implicated in sperm function during fertilization. The level of SP22 on rat sperm has been shown to correlate highly with fertility when conventional semen analysis parameters did not. Additionally, antibodies raised against this protein have been shown to inhibit IVF in rats. In rats and hamsters this protein is localized to the equatorial segment, consistent with a role in sperm-egg interaction, and to the tail of the sperm. In human sperm this protein has been reported to be localized only to the sperm tail. Herein, we sought to determine the localization of SP22 in a series of human sperm samples and to associate localization patterns with the quality of the sperm sample. Human sperm were obtained from 26 men of unknown fertility and semen parameters (motility, viability and morphology) were evaluated. Subsequent to this, 15 samples were washed, fixed, and immunostained; 11 samples were subjected to Isolate? treatment prior to fixing for immunostaining. Significant numbers of sperm in all samples (34 to 80%) showed staining in the equatorial segment and/or the proximal portion of the neck. Isolate? treatment resulted in a statistically significant increase (from 56 to 66%) in SP22 equatorial segment and proximal neck staining. A low level of SP22 staining was seen on the tails of almost all sperm. Consistent with the previous studies in rats, there was no apparent correlation between SP22 staining and conventional semen analysis parameters. In summary, the localization of SP22 on human sperm is consistent with the role of this protein in fertilization and fertility indicated in animal studies. Correlation of SP22 to fertility in humans awaits a prospective clinical study in infertile men. Supported by a grant from US EPA.
Abstracts – Poster Session 3/4

P3/4 – 023
INFLUENCE OF ANGIOTENSINS ON HUMAN SPERM MOTILITY
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In previous experiments angiotensin II (AI), in contrast to angiotensin I (AII), was shown to induce human sperm acrosome reaction. The purpose of the present study was to investigate the influence of AI and AII on sperm motility and different motion parameters. Therefore, 17 ejaculates from patients of our Andrological Department and 22 ejaculates from young and healthy donors were examined. After glassworen filtration and one washing step in IITF (1% HSA), sperm concentration was adjusted to 20 mio/ml. Spermatozoa were treated with AI (10-6 mol or 10-5 mol) or AII (1 nm or 100 nm) for 15min and 60min. The percentage of immotile, local motile and progressively motile spermatozoa and some special motion parameters, including different velocity parameters were measured by a computer assisted sperm motion analyser after the different incubation times and a final washing step. The strongest stimulatory effect on motility was seen in patients as well as in donors by incubating spermatozoa with 1nm AII for 60min (65.6±/-2.7% vs 47.9±/-2.3%; p<0.001), remaining also after the washing procedure. A significant positive influence on the velocity parameters (VCL,VSL,VAP) and the linearity was seen. This effect was markedly inhibited by the AT1-receptor-selective antagonist losartan (5x10-5 mol), whereas PD123319 (AT2-receptor-blocker) showed no significant effect. With immunocytochemistry this result was confirmed showing AT1-receptor immunoreactivity in the flagellum and midpiece and AT2-receptor immunoreactivity in the acrosomal region of human spermatozoa. Thus AII may stimulate sperm motility via activation of the AT1-receptor. In contrast, the AT2-receptor may play a regulatory role in the induction of the acrosomal reaction.

P3/4 – 024
PURIFICATION OF HUMAN SEMINAL PHOSPHOLIPID-BINDING PROTEINS CLOSELY RELATED TO THE MAJOR PROTEINS OF BOVINE SEMINAL PLASMA.
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The major proteins (BSA-AI/A2, BSA-A3 and BSA-30-kDa) of bovine seminal plasma (BSP) are a family of phospholipid- binding proteins and they play an important role in sperm capacitation. Analogous proteins of this family have been isolated from boar and stallion seminal plasma and characterized. Our previous study has shown that this family of protein specifically interacts with the low- density fraction (LDF) of hen's egg yolk and that these proteins could be isolated along with LDI by ultracentrifugation. In this study, we provide the evidence for the existence of analogous phospholipid-binding proteins in human seminal plasma. The alcohol precipitates of human seminal plasma were incubated with LDI and the LDF with bound proteins were reisolated by ultracentrifugation. Analogous proteins were finally purified by p-aminophenyl phosphoryl-choline-Agarose and gelatin-Agarose chromatographies. The purified proteins were analyzed following SDS-PAGE and immuno blotting using polyclonal antibodies against BSA-AI/A2, -A3 and -30-kDa proteins. These results showed in the adsorbed fractions, the presence of BSP protein analogs in human seminal plasma. The radioimmunoassay data indicated that the BSP proteins analogs are present in very low concentration in human seminal plasma (0.1 µg/g for BSA-AI/A2 cross-reacting proteins) but can be largely enriched by the purification (up to 20,341-fold). The human proteins isolated by this protocol exhibit: binding properties, molecular weights and immunological properties similar to those of BSP proteins. (Supported by CIHR)

P3/4 – 025
TWO NOVEL METHODS FOR GERM CELL TRANSPLANTATION (GCT)
Nik Sovikitis*, Yasu Yamamoto, Itaru Miyagawa, Yonago, Japan

OBJECTIVES: We evaluated the outcome of novel methods for GCT into the seminiferous tubules. METHODS: STs from cryptorchid hamsters (arrest at the primary spermatocyte stage) were placed subcutaneously (in contact with the super surface of the rectus muscle) in 13 nude rats (group A). The rat subcutaneous tissue and the skin over the hamster STs were removed and replaced by a rat scrotal graft. Thus an abdominal scrotum (AS) containing hamster STs was created in each rat. Thirteen different nude rats (group B) underwent removal of some STs from their left testicles. Then STs from cryptorchid hamsters were placed into the left testicles under the unica albumeinaj in contact with the rat's STs. Thus each rat of group B contained both hamster and rat STs into its left testicle. Three times a week vascular endothelial growth factor was injected into the donor STs in both groups A and B. Five months post-GCT the ASs of group A rats and the left testicles of group B rats were open. Fractions of donor STs were processed for transmission electron microscopy (TEM), or vascular stain, or mincing techniques for dispersion of germ cells. RESULTS: TEM showed that 2 rats of group A and 3 rats of group B were positive for hamster round spermatids and hamster spermatozoa, respectively. Vascular stain showed sufficient vascularization in the hamster STs in the latter 5 animals. When hamster spermatozoa from group B rats were processed for ICSI a fertilization rate (FR) of 6% was achieved. CONCLUSIONS: Placement of donor STs into a recipient testicle or an AS can serve as novel methods for GCT and result in vascularization of the donor STs and partial or total restoration of the donor spermatogenesis within the recipient testicle or AS.

P3/4 – 026
ISOLATION AND CULTURE OF ENRICHED POPULATION OF TYPE A-SPERMATOGONIA FROM PREPUBERTAL BULL TESTES.
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This study was aimed to isolate, purify and culture bovine type A-spermatogonia. Testes from 3 to 7 months old calves were used to isolate germ cells using a two-step enzymatic digestion. After isolation a cell suspension was obtained containing about 25% A spermatogonia, which was further enriched by differential plating and separation on a discontinuous percoll gradient. Finally, 65-87% pure type A -spermatogonia could be obtained. Cells were cultured in MEM or KSO in and different concentrations of FCS for 2-4 weeks at 32°C or 37°C. Culture in MEM resulted in more viable cells and more proliferation than in KSO and better results were obtained at 37°C. Viability of cells after isolation was about 90%. After one week of culture in the absence of serum only 20% of the cells were alive. However, in the presence of 2.5% FCS about 80% of cells were alive and proliferating. Higher concentrations of FCS, only enhanced viability and numbers of somatic cells. In long term culture, spermatogonia continued to proliferate and eventually A-spermatogonial colonies were formed. Two types of colonies were observed. Large round colonies consisting of single, c-kit negative A spermatogonia and smaller radial colonies that were more numerous and consisted of single cells and small groups of cells connected by intercellular bridges. Most of the cells in the radial colonies were c-kit positive. The data suggest the existence of two types of spermatogonial stem cells. One that in culture only carries out self-renewal and one that also forms differentiating daughter cells.
Abstracts – Poster Session 3/4

P3/4 – 027
CHARACTERIZATION OF SPERMATOGONIAL TYPES IN MOUSE SPERMATOGENESIS UTILIZING SEMI-THIN SECTIONS OF PERFUSED TISSUE.
H Chiariini-Garcia* and LD Russell. Department of Physiology, Southern Illinois University, Carbondale, IL, 1Department of Morphology, Federal University of Minas Gerais, Brazil.

Spermatogonia kinetics in the mouse is as follows: Aγ, Aβ, Aν, Aθ, Aη, Aξ. In and B, with the Aζ to Aξ transition lacking a mitotic division. Some reports have used whole-mounted seminiferous tubules or paraffin embedded H&E sections to characterize the cells from A1 through A4, although the documentation of the morphology of these cell types is poor. In the present study, over 1600 digitized images of Type A gonial cell types were taken of well-fixed, vascular-perfused tissue embedded in epoxy to determine if gonial cell types could be characterized in the mouse at high resolution. High magnification images were taken of all Type A gonias in seminiferous tubules at all stages of the cycle. A minimum of 150 images was taken for each stage. The literature suggests that 8-15% of gonias in most stages could be characterized as Aγ, Aβ, and Aν cells. We found a similar proportion of cells in randomly taken light micrographs. Their nuclei displayed an uneven (splotchy) texture among numerous other more minor characteristics such as the presence of nuclear vacuoles. Aη through Aξ gonias displayed a more even textured nucleus. Heterochromatin along and within the nuclear envelope gradually increased in these cells as they proceeded from Aη through Aξ. The heterochromatin of type Aν and Aξ gonias was distinctive, allowing their differentiation. This study defines the morphological characteristics of Aη, Aν, and Aξ cells, separate from more mature gonias. (Support: Latin American Fellowship from the NIH to HC-G; NIH HD35494 to MDG & LDR).

P3/4 – 028
THE SPATIAL ORGANIZATION OF SPERMATOGENESIS IS REGULATED BY THE STAGE-RELATED TOPOGRAPHY OF TYPE A SPERMATOGONIA IN THE MOUSE

Type A spermatogonia were examined in mouse seminiferous tubules to determine if their position was random. In perfused-fixed seminiferous tubules, the position was classified as either facing the interstitium or facing other tubules. Camera lucida drawings were made of these regions of seminiferous tubules and Type A gonias were drawn in 5 of 12 stages. Data were expressed as a ratio of the percentage of gonias in a particular region to the percentage of the tubule periphery comprising that region. For example, if 50% of the gonias were classified to 50% of the tubule that faced a particular region, the ratio would be 1.00. Ratios higher than 1.00 indicated that gonias were located preferentially; those lower than 1.00 indicated they were absent. At stage V, Type A gonias were randomly positioned within seminiferous tubules; at stage VI most moved to face the interstitium and remained in this position until after stage VII. Although Aη, Aν and Aξ gonias remained near the interstitium, the Aζ-Aξ distribution gradually became random. The non-random distribution of primitive gonias prior to their division to form Aη-Aξ cells suggests that they position themselves at evenly spaced sites around the seminiferous tubule. As the progeny of Type A cells divide, they fill in the gaps between the evenly spaced primitive gonias. An even distribution of young germ cells around the base of the tubules insures that spermatogenesis is conducted uniformly around the seminiferous tubule. These data suggest that the regulating factor for gonias emanate from the interstitium. (Support: Latin Am. Fellowship-NIH, HC-G; NIH HD35494, MDG & LDR)

P3/4 – 029
TELOMERAZE IMMORTALIZED SPERMATOGONIAL CELL LINES.
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The establishment of an in vitro germ-cell line would greatly facilitate our ability to elucidate the molecular mechanisms regulating spermatogenesis. We have obtained cell lines by over expressing telomerase in highly purified type A spermatogonia. Telomerase functions to prevent cells from replicative senescence and maintains cell division. Recent studies found that introducing telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, into certain cell types can expand their lifespan and even immortalize them. Telomerase is highly expressed in various types of stem cells including the male germ-line stem cell. Furthermore, its activity is gradually lost during differentiation. Thus, it has been suggested that telomerase may be necessary to maintain the proliferative potential of stem cells. Based on the above observations, we attempted to immortalize male germ-line stem cells from 6-day-old mice by introducing mTERT cDNA with a retrovirus vector. We obtained several cell lines that possess a number of morphological and biochemical characteristics of type A spermatogonia. First, all of these cell lines express Dazl, a germ cell specific RNA-binding protein. Then we checked for the expression of c-kit and GFRα-1. In normal testis, c-kit is expressed in most of the type A spermatogonia, but GFRα-1 is believed to be expressed only in spermatogonial stem cells. Using Western blot analysis, all our cell lines were found to express both c-kit and GFRα-1. Interestingly, one of the cell lines expressed low levels of c-kit and high levels of GFRα-1. This latter cell line may contain a higher proportion of spermatogonial stem cells. Using GFP as a reporter gene driven by an acrosome promoter, the cells expressed GFP when cultivated in the presence of retinoic acid. In summary, over expressing telomerase may be a useful method to obtain spermatogonial cell lines.

P3/4 – 030
QUANTIFICATION OF HUMAN TELEOMERAZE REVERSE TRANSCRIPTASE MRNA EXPRESSION IN TESTICULAR TISSUE OF INFERTILE PATIENTS
Mark Schrader, Markus Müller, Rüdiger Heicappel, Bernd Straub, Kurt Miller, Berlin, Germany

Introduction and Objectives: Telomerase is a ribonucleoprotein detectable in germ, stem and tumor cells. In male germ cells, it plays an essential role in transmitting full-length chromosomes to progeny. Human telomerase reverse transcriptase (hTERT), a major component of the enzyme, was identified in testicular tissue as a highly specific marker of gametogenesis. The aim of this study was to determine whether quantification of hTERT mRNA expression can predict the presence of haploid germ cells in testicular biopsies. Methods: hTERT mRNA expression was quantified in 39 testicular tissue specimens by fluorescence real-time RT-PCR. This was paralleled by conventional histological workup, wet preparation and semithin sectioning preparation. 18 biopsies were taken from patients with Sertoli-cell-only syndrome (SCOS). 19 from pat. with maturation arrest (MA) (Johnsen Score 3-5) and 22 from pat. who had obstructive azoospermia with normal spermiogenesis (OA) (IS 6-10). Results: There were 130 (± 23.8 standard deviation) mean hTERT mRNA copies normalized to 1000 copies of PBGD mRNA in tissue specimens with full spermatogenesis and 73 (± 9.3) in those with MA, while only minimal hTERT expression (1.5 ± 0.5) was detectable in SCOS specimens. Conclusions: Our investigations show that the level of hTERT mRNA expression in testicular biopsies is correlated to specific stages of gametogenesis. Quantification of hTERT mRNA by fluorescence real-time RT-PCR is thus a highly sensitive and highly specific new technique that enables a new molecular-diagnostic subclassification of gametogenic disorders and opens up interesting perspectives in detecting focal spermatogenesis in Sertoli-cell-only specimens.
P3/4 – 031
THE REGULATION OF TELOMERASE ACTIVITY AND TELOMERE LENGTH IN THE STERILE RAT'S TESTIS. I Oh'Har*a, TT Tomura,b, Department of Biochemistry, Kinki University School of Medicine, Osaka-Sayama, Japan. The sterile rats characterized by curled whiskers and coat atrophy were developed from Wister-Kyoto strain by Sasegawa in 1990. Both sexes of the strain are sterile and demonstrate aplastic atrophy in the gonads of the adulthood. The sterile rats were different for Zitter rats in genetic aspects. To investigate the cause of male sterility, the tests of different ages were studied for any hormonal, histo- and biochemical changes. Microscopic observation revealed that spermatogonia, pachytene spermatocytes, and Sertoli cells appeared in seminiferous tubules of the sterile rats even after 4-week-age. These aspects seems to depend on the abnormality of cell differentiation. The cell death and wasulated cell growth were predicted based on the loss of chromosome integrity. To study the molecular mechanism during cell differentiation, telomere shortening and telomerase activity were determined in sterile rats. We assayed for telomere length and telomerase activity in rat's tests of different ages. Telomerase activity were determined using the PCR based "TRAP" assay method. Telomerase activity and telomere length were similar between S/1 and sterile rat within 4 weeks of age, whereas telomerase activity were differed in adults r.t.

P3/4 – 032
DNA DAMAGE IN SUBSETS OF HUMAN SPERMATOZOA AT DIFFERENT STAGES OF MATURATION. M Oller*a, M Gil-Guzmanb, RK Sharma*, MC Lopez*, K Larson*, D Eveson*, AL Steinlen*, A Terheyden*, A Agarwal*, and GB Alini*, Department of Obstetrics and Gynaecology, Beth Israel Deaconess Med Ctr, Harvard Medical School, Boston, MA;* Urological Institute, The Cleveland Clinic Foundation, Cleveland, OH;* Dept of Chemistry and Biochemistry, South Dakota State University, Brookings, SD. DNA damage in human spermatozoa has recently been identified as an indicator of poor pregnancy outcome in vitro and in vivo fertilization. However, the mechanism by which this damage takes place has not yet been elucidated. ROS has been shown to induce sperm DNA damage in vitro. In this study, ROS production and DNA damage were determined in human sperm at different stages of maturation. Semen samples were obtained from healthy doners (n=16) and infertility patients (n=32) and classified as having normal (NSP) or abnormal (ASP) semen parameters, according to WHO criteria. Four sperm subsets were isolated from semen samples by a three-step (47.70±0.9%) Isolate density gradient ROS production was measured by the standard chemiluminescence assay. The composite value of the logarithm of ROS levels in a given fraction multiplied by the relative concentration of sperm in that fraction is designated as relative ROS levels. DNA damage was measured using the sperm chromatin structure assay and expressed as %COMP*. The results are shown in the table below (mean±SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
<th>Fraction 3</th>
<th>Fraction 4</th>
<th>Rel ROS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNRSP</td>
<td>95.9±10.5</td>
<td>94.5±15.5</td>
<td>93.5±17.7</td>
<td>92.5±15.5</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>ISP</td>
<td>95.2±18.5</td>
<td>95.4±17.6</td>
<td>95.6±15.9</td>
<td>95.7±16.6</td>
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</table>

P3/4 – 033
ALTERED CREM LOCALIZATION IN SPERMAGENIC CELLS IN TESTOSTERONE (T)-TREATED SPINAL CORD INJURED (SCI) RATS. HFS Huang*a, R Anesetitib, CA Molina, B west, SL Wang, JE Ottenwellera, LM Pogach, V.A. Medical Center, East Orange and UMD-New Jersey Medical School, Newark, NJ, USA. The current experiment examined does effects of exogenous T given as T-filled silastic capsules (TC) on spermatogenesis in SCI rats, and their relationship with cellular distribution of CREM. Implantation of 2-20 cm TC resulted in dose-dependent, biphasic changes in testicular T levels and spermatogenesis in SCI rats. Specifically, 2 cm TC induced regression of spermatogenesis to an extent that was far more severe than those in intact or hypophysectomized (HPX) rats, regardless of T regimens. While 3 cm TC was sufficient to maintain early spermiogenesis, it failed to support the final steps of spermatid differentiation. Complete spermatogenesis was maintained in those SCI rats given 5-20 cm TC implants. Immunostaining revealed that CREM was present in young spermatids and pachytene spermatocytes in sham control rats; such pattern was not affected by SCI. Normal pattern of CREM localization was maintained in SCI rats given 1, 10 or 20 cm TC implants. In contrast, CREM in young spermatids and pachytene spermatocytes were absent or greatly reduced in SCI rats given 3 or 5 cm TC implants. In these animals, CREM was localized in young spermatids and spermatogonia. These changes in CREM distribution in spermatogenic cells were associated with parallel changes in CREM levels. In conclusion, SCI resulted in changes in the responsiveness of spermatogenesis to exogenous T, and these effects were associated with altered CREM distribution in spermatogenic cells. The precocious presence of CREM in spermatogonia and young spermatids and the lack of in spermatids and pachytene spermatocytes in SCI rats given 3 or 5 cm TC implants and had abnormal spermiogenesis suggest a possible cause-effect relationship between these changes. Since these changes were different from those seen in HPX or intact rats given comparable doses of T, they might result from the interaction of SCI and changes in testicular microenvironments elicited by specific doses of T.

P3/4 – 034
ISOLATION AND CHARACTERIZATION OF HAPLOID GERM CELL SPECIFIC NOVEL CDNA; TESTIS-SPECIFIC HOMOLOGUE OF Succinyll CoA: 3-OXO ACID COA TRANSFERENCE ACE (SCOT-T). M. Koga*a, J. Kohroib, H. Tanaka*, K. Yomogida, M. Nozaki*, H. Ohta*, N. Iuchi*, Y. Nakamura*, M. Yamanaka*, A. Tsujimura*, M. Takeyama*, H. Nojima*, K. Matsumiya*, A. Okuyama*, and N. Mishima*, Department of Urology, Osaka Central Hospital, Department of Urology, Osaka University Graduate School of Medicine, and Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. spermiogenesis includes very interesting phenomena of morphological changes, specific gene expressions and transcriptional regulations. To understand the molecular mechanism of haploid germ cell differentiation, a subtracted mouse testicule cDNA library was prepared to concentrate cDNAs specifically expressed in haploid germ cells. From this library, we isolated a novel cDNA, which was a homologue of succinyll CoA: 3-oxo acid CoA transferase (EC2.3.3.5), expressed specifically in testicular haploid germ cells, and named scot-t. Northern blot analysis, in situ hybridization and Western blot analysis demonstrated the unique expression of the mRNA concomitant with rapid translation exclusively in late spermatids. Scot-t protein was detected first in elongated spermatids at step 8 or 9 as faint signals and gradually intensified during spermiogenesis. It was also detected at the mid piece of spermatozoa by immunohistochimistry. From these data, we supposed that the autologue of scot-t was also isolated from human testis library. Succinyll CoA: 3-oxo acid CoA transferase is a key enzyme for energy metabolism of ketone bodies. Scot-t might play some important roles in energy metabolism of ketone bodies in the sperms of human and mouse.
P3/4 – 035

RATE OF HOMELOGOUS CHROMOSOME PAIRING IN SPERMATOCYTES MAY PREDICT COMPLETION OF THE SPERMATOGENESIS PROCESS IN TESTES OF AZOOSPERMIC MEN

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The rate of bivalent formation of four pairs of chromosomes – X-Y, 9, 15, and 18 – was correlated with the presence and amount of spermatozoa in the testes of 25 azoospermic men. Three biopsies from each testis were taken to extract spermatozoa for the intracytoplasmic sperm injection procedure. In addition, one sample from each testis was used for histological definition, spermatozoa count and for detection of chromosome pairing in spermatoocytes. Bivalent formation was detected by the fluorescence in situ hybridization technique. Spermatozoa in at least one biopsy were detected in 20 men. A significantly higher rate of pairing of all homologous chromosomes was found whenever spermatozoa were detected. The rate of bivalent X-Y was found to be the most sensitive predictor for detection of spermatozoa, with a cut-off value of 47%. It was also the best predictor for the number of spermatozoa in the minced sample (R²=34%, P=0.002) and for the number of mature spermatids per tubule in the histological section (R²=36%, P=0.002). Thus, it may be concluded that the rate of X-Y bivalent formation in spermatoocytes may predict the presence and amount of spermatozoa in the testicular tissue of azoospermic men.

P3/4 – 036

DNA DAMAGE IN GERM CELLS IS NOT FULLY REPAIRED DURING SPERMATOGENESIS

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Previous studies have shown that testicular heating adversely affects fertility. This study examines the effect of heating on the genetic integrity of germ cells in the testis and mature sperm in the epididymis. Adult male mice were anaesthetised, subjected to scrotal heating at 42°C for 30 minutes and allowed to recover. Testes and epididymes were retrieved at 1 hour (h), 2h, 4h, 6h, 24h, 7 days (d), 14d, 21d, 24d, 28d and 32d post-heating (PH). The expression of a number of heat and oxidative stress markers and the presence of apoptotic cells in the testis and epididymis were studied. DNA integrity of mature sperm from the epididymis was studied using a modified Comet assay. Reduced expression of Cirt and translacation of HSP105 from the cytoplasm to the nucleus of germ cells, increased apoptosis and elevated expression of HO1 in Sertoli cells indicated that the testes had been affected by heating. Mature sperm located in the epididymis at the time of heating (1, 2, 4 and 6h PH) was found to have higher levels of DNA damage than that of control (anaesthetic only) animals. This DNA damage increased over time, reaching a peak at 4h PH. Mature sperm collected 24h PH (late spermatids at time of heating) also showed a slight increase in DNA damage compared to controls. Mature sperm originating from Stage 11 (7d PH) and Stage 2-3 Spermatids (14d PH) exhibited levels of DNA damage comparable to the controls indicating that these cell types are not susceptible to heat damage. However, levels of DNA damage began to increase 21d PH (mid pachytene), continuing through 24d (early pachytene) and 28d (leptotenes). These results suggest that mature sperm in the epididymis are susceptible to heat-induced DNA damage and that DNA damage induced in the testis may not be fully repaired during spermatogenesis.

P3/4 – 037

cDNA CLONING AND SEQUENCE ANALYSIS OF PROSTAGLANDIN D SYNTHASE IN HUMAN TESTIS

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Objectives: Lipocalin-type prostaglandin D synthase (L-PGDS) is a dual function protein, able to catalyze the transformation of PGH2 to PGE2 and transport lipophilic substances. It was reported that L-PGDS in human testis may be closely related to spermiogenesis. We have not found L-PGDS cDNA sequence in human testis yet. The objective of this paper is to clone and sequence the L-PGDS cDNA in human testis.

Methods: Human testis RNAs are extracted with Trizol kit then transcribed reversely and amplified by PCR using the primers from L-PGDS cDNA in the brain, and the PCR products are purified and sequenced.

Results: L-PGDS cDNA in human testis can be cloned with specific primers of L-PGDS cDNA in human brain, but L-PGDS in rabbit and mouse can’t be cloned with the same primers. L-PGDS cDNA in human testis consists of 593 nucleic acids and encodes 190 amino acids. The nucleotide sequence of L-PGDS cDNA in human testis has 99.5% homology with gene sequence encoding L-PGDS in human peripheral blood lymphocytes and 96.0% with L-PGDS cDNA in human brain. The homology of corresponding amino acid sequences are 100% and 94.2% respectively.

Conclusions: The explanation of L-PGDS cDNA and derived amino acid sequence in human testis is very important to further explore characteristics of L-PGDS protein and its roles in male reproduction.

P3/4 – 038

CONSTRUCTION AND IDENTIFICATION OF RECOMBINANT PLASMIN EXPRESSING HUMAN TESTIS PROSTAGLANDIN D SYNTHASE

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Objectives: To construct recombinant plasmin expressing human testis lipocalin-type prostaglandin D synthase (hL-PGDS) efficiently.

Methods: We acquired nucleotides sequence encoding L-PGDS by PCR amplification with L-PGDS cDNA template which was certified to be human L-PGDS by sequence analysis. The nucleotide sequence was digested with restriction endonuclease BamH I and EcoR I, then inserted into pGEX-2T which was also digested with the same endonuclease. The recombinant plasmid pGEX-2T/hL-PGDS was further transferred to competent Escherichia Coli IM103. The resultant was inoculated on LB medium plate containing ampicillin. After 12-16h at 37°C, the ampicillin resisting bacteria clones were picked up and inoculated to liquid LB medium containing ampicillin. Amplified for 12-16h under the condition of 37°C and 160 r/min, the plasmid DNA were isolated and purified for identification by PCR and digestion with BamH I and EcoR I.

Results: 109 ampicillin resisting bacteria clones were acquired. Among them, 10 clones containing recombinant pGEX-2T/hL-PGDS were identified by PCR and digestion with BamH I and EcoR I.

Conclusions: The construction and identification of recombinant expression plasmid pGEX-2T/hL-PGDS lay solid foundations for the expression and purification of recombinant antigen and the preparation of monoclonal antibodies to this antigen.
P3/4 - 040

INSIG3 GENE MUTATIONS NOT RELATED TO HUMAN CRYPTORCHIDISM

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Introduction. Cryptorchidism is a frequent congenital anomaly and an
important cause of infertility and testicular cancer. The etiology is still
unclear and genetic causes may be involved. INSIG3 has been proposed as
putative gene for cryptorchidism, since INSIG3/-/- mice have bilateral abdomi-
nal testes due to alteration of gubernaculum development. Materials and
methods. By means of semen analysis, hormonal levels for FSH, LH and
testosterone, ultrasound scanning of the testes and testicular FNAC we
selected 65 ex-cryptorchid infertile patients. Only 46, XY patients with
a normal analysis for Yq microdeletions were included. Thirty-two patients
had a history of unilateral and 33 of bilateral cryptorchidism. 30 control
subjects were studied. Heteroduplex and sequence analyses were per-
formed. Results and discussion. Sequencing revealed 4 GA substitutions at
position 27, 96, 126 and 176, corresponding to codon 9, 32, 42 and 60.
The former three did not change amino acid, whereas the 178G→A sub-
stitution changed alanine to threonine (A60T). Mutation rate was 37% in
ex-cryptorchids and 45% in non cryptorchids. With regard only to the
A60T mutation, this variation was found in comparable distribution in
both patients and controls. Therefore, these variants most likely represent
neutral polymorphisms not related to phenotype. Prevalence of mutation
in the group of ex-cryptorchords was not different considering unilateral vs
bilateral cryptorchidism, among patients with different degree of testicu-
lopathy or between patients with and without associated malformations.
These results apparently suggested that mutations of INSIG3 gene are not a
frequent cause of cryptorchidism.
P3/4 - 043

LOCALIZATION OF DAZ mRNA IN HUMAN TESTIS USING REVERSE TRANSCRIPTION IN SITU PCR TECHNIQUE (RT-ISPCR).
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Normal course of spermatogenesis reflects appropriate expression of specific genes in spermatogenic cells. 10-15% of men diagnosed with the so-called undefined infertility was found to have DAZ gene deletions. The present study was aimed to determine the distribution of DAZ mRNA in human testis obtained from five fertile organ donors and four patients with the deletion of DAZ gene, using RT- ISPCR method. The reaction products were detected within entire epithelium of seminiferous tubule. The DAZ mRNA was found mostly in primary spermatocytes and spermatids. Besides, the tubules with irregular distribution of DAZ mRNA were found. In some regions the DAZ mRNA was detected in all kinds of germative cells, from spermatogonia to spermatids. There were the regions in which DAZ transcript was observed in primary spermatocytes and spermatides, whereas the minor- or no staining was observed in spermatogonia. In most cases strong accumulation of DAZ transcript was specific for primary spermatocytes. Occasionally the DAZmRNA was found in spermatocytes. The products of RT-ISPCR have not been found within lamina propria of tubules and interstitium. The results indicate that expression of DAZ gene starts in spermatogonia and that distribution of DAZ mRNA most likely correlates with the stage of the human seminiferous cycle. In all patients with the deletion of DAZ gene the reaction products were not observed. The analysis of DAZ gene expression allows the determination of the functional stages of seminiferous tubules and can be a useful tool in search for cause of male infertility.

P3/4 - 044

DYNAMICS OF TESTICULAR HORMONE-SENSITIVE LIPASE (HSL) DURING POST NATAL DEVELOPMENT IN GUINEA PIG (GP).
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HSL protein levels and enzymatic activity were respectively measured by densitometry of immunoreactive bands obtained in Western blots using affinity-purified polyclonal antibodies against recombinant rat HSL and by spectrophotometry in seminiferous tubule (STF) and interstitial tissue (ITF) enriched fractions from 6-, 11- and 21-day-old and adult GP testes. The presence of HSL was studied in subcellular fractions of STF from adult testes. A HSL immunoreactive band of 104-kDa was detected in STF and ITF, the expression of which increased with development. Two additional bands, of 110- and 120-kDa were found in the STF of adult testes. The 104-, 110- and 120-kDa immunoreactive bands were detected in the lysosomal fraction from STF of adult testes. HSL activity increased during development and was positively correlated with free and esterified cholesterol ratios quantified by gas-liquid chromatography in STF and ITF of each age group, but not with triglyceride levels. Immunolabeling localized HSL in elongating spermatids and Sertoli cells. The detection of the 104-, 110- and 120-kDa immunoreactive bands in STF of adult testes localized to lysosomes suggests that HSL may originate in part from germ cells and be partly imported in Sertoli cells for processing. HSL protein levels and enzymatic activity in ITF and STF were positively correlated with serum testosterone levels suggesting a role of the enzyme in spermatogenesis. Support NSERC to RMP and to MLV.

P3/4 - 045

CAVEOLIN AND FLOTILLIN CONTENT OF RAT TESTIS PERITUBULAR MYOID CELLS AND THEIR DETERGENT-INSOLUBLE GLYCOSPHERINGOLIPID ENRICHED MEMBRANE MICRODOMAINS.
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Recently we reported that primary cultures of rat Sertoli cells possess detergent-insoluble glycosphingolipid (DIG) enriched membrane microdomains, i.e. rafts. These microdomains, based on their hydrophobic character and unique lipid content, can be isolated due to their insolubility in 1% Triton X-100 and their low buoyant density. Rafts are found in a wide variety of cells and include microsponyocytic vesicles, known as caveolae, as well as other non-caveolar microdomains. The occasional observation of low caveolin levels in Sertoli cultures, otherwise negative, that correlated with peritubular myoid cell contamination, led us to investigate the caveolin and flotillin content of highly purified PMC cultures. We report here that PMCs, in contrast to Sertoli cells, contain high levels of both caveolin and flotillin. In addition, DIG fractions can be isolated from PMCs, and they contain a major enrichment in caveolin and flotillin. Primary PMC cultures were grown for four days and then passed into twenty 75 cm² culture flasks. The identity of PMCs was confirmed using anti-smooth muscle actin immunofluorescence microscopy. PMCs were grown to confluence and DIG fractions harvested. These DIG fractions were evaluated for caveolin and flotillin content following SDS-PAGE, western blotting, immunostaining, and ECL. Immunofluorescence microscopy of cryostat-sectioned testis revealed that caveolin was absent from Sertoli cells but present in the PMCs surrounding the seminiferous tubules. These data suggest that PMCs contain caveolin concentrated in DIG fractions. Further, microsponyocytic vesicles of the PMCs should be examined for caveolin content and their possible identity as caveolae.

P3/4 - 046

The Localization of IGF-1 By Immunohistochemical in Boar Testes During Prepuberty
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Studies have shown that prepuberal growth hormone (GH) supplementation to boars advances the time of onset of spermatogenesis. Since insulin like growth factor-I is known to be an intermediate of GH action, the objective of this study was to investigate the occurrence and distribution of insulin-like growth factor-1 (IGF-1) in the testis of prepuberal boars. Three boars at a time were castrated under Ketamine/Rompun anesthesia every 10 days between the ages of 10 and 120 days (total ~16 boars). One testis from each bear was perfused with Bouin's fixative, dehydrated, and embedded in paraffin. An immunohistochemical method for IGF-I using the UB3 anti-hIGF-1 antibody from the National Hormone and Pituitary Program as primary antibody and the avidin-biotin complex for visualization was developed. Optimal staining of the hydrated sections was obtained after 2 hours incubation with primary antibody at a 1:100 dilution. The Leydig cells were positive for IGF-I at all ages but weaker staining occurred in 10 day-old pigs. The gonocytes were diffusely stained at younger ages. Beginning in 30 day old pigs and cumulating in 70-90 day old boars, the stained area appeared as a well defined crescent shaped structure localized near the nuclei, similar to the localization of gonocyte mitochondria. Whether IGF-I is associated in the mitochondria function is not known. The Sertoli cell cytoplasm featured streaks of staining at 80-90 days and became diffusely stained from 100 days of age. It is concluded that IGF-I changes in intensity and form during the peripuberal period in boar gonocytes and Sertoli cells and may, in part, be a mediating factor of GH action in spermatogenesis in the prepuberal gonocytes and Sertoli cell cytoplasm might be a mechanism by which GH influences onset of spermatogenesis aside from the known stimulation by IGF-I on Leydig cells.
P3/4 - 047
MODULATION OF GLUTATHIONE LEVEL IN RAT TESTICULAR MITOCHONDRIA BY HYPOTHYROIDISM.

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Thyroid hormones are considered to be physiological modulators of tissue oxidative stress. Therefore, any alteration in thyroid hormone level will not only affect the general metabolism but also oxidative stress status of the tissue. Thiols compounds are known to play a crucial role in protecting tissues from oxidative stress and testis is reported to have relatively high level of GSH. In the present study effect of hypothyroidism on GSH and GSSG status of the mitochondria is investigated in order to find out the role of thyroid hormone on testicular mitochondria. Hypothyroidism in adult rats was induced by providing propyl-thiourica in drinking water for 30 days. Hypothyroidism resulted in elevation of hydrogen peroxide level in the mitochondria of testis. This is accompanied with a significant decline of reduced glutathione content of the mitochondria of testis. Although testicular mitochondrial fraction exhibited an elevation in GSSG level, the change was not statistically significant. However, the ratio GSSG/GSH in mitochondria of hypothyroid testis is significantly higher than the control rats suggesting induction of oxidative stress in testis by hypothyroid state. Although total glutathione peroxidase level of mitochondria of testis did not alter by hypothyroidism a significant increase in non-selenium dependent glutathione peroxidase was recorded. Similar increase in glutathione reductase level in mitochondria of hypothyroid rats was noticed. The results indicate that hypothyroidism can induce oxidative stress in testis by modulating glutathione level in its mitochondria.

P3/4 - 048
HORMONAL ALTERATIONS AND SPERMATOGENIC ARREST UNDER CAFFEINE ADMINISTRATION IN RATS.

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The effect of caffeine (CA), methylxanthine alkaloid presenting in tea, coffee, cola soft drinks and other over-the-counter drugs, at a certain dose of 60 mg/kg b.w. 3,6 & 9 hrs (single doses) and 14, 21 & 28 days (chronic doses) after intraperitoneal injection was evaluated on serum Testosterone (TES), Prolactin (PRL), DHEA-S, FSH and LH levels, using RIA method, and also on Spermatogetic array in testicular tissue. Animal groups were: control, sham-operated and test. CAF caused a time-bound marked diminution (P<0.05, ANOVA) in the serum levels of TES, PRL & DHEA-S in both single and chronic doses. The relationship between CAF impact on adenosine receptors and PRL role as a helper hormone to LH surge on Leydig cells was discussed. Elevated levels of FSH (only after 6 hrs) and LH (after 3 hrs. and 21 days) were found significantly. We next examined the CAF induced changes in histarchitecture of testes. It was found that CAF caused time-dependent spermatogenic arrest being more pronounced in 28 days drug (tread) groups: reduction in number of spermatogonia B & spermatocyte I. The seroill-germ cell association was also disrupted in many tubules. Further, the sperm count in epididymis and seminiferous tubules diameter were adversely affected. The results suggest that CAF consumption in a prolonged duration can alter the pituitary - gonadal axis and affect the male fertility as well.

P3/4 - 049
A NOVEL MUTATION OF ANDROGEN RECEPTOR ENCODING GENE IN PATIENT WITH PARTIAL ANDROGEN INSENSITIVITY SYNDROME

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Molecular genetic analysis of androgen receptor encoding gene was done in 4 Lithuanian patients in Righospitalet, Copenhagen. 1.18 years old girl with complete androgen insensitivity syndrome (CAIS). 2. Her mother, 46 years old women. 3.14 years old girl (46,XY) with severe virilization during the puberty. 4.14.5 years old boy with partial androgen insensitivity syndrome (PAIS). He was referred to the Institute of Endocrinology due to gynecomastia (Tanner stage B4) and female body proportions. His pubertal stage was T3G3, but no axillary hair, karyotype - 46XY. The boy had male external genitalia with normal penis and scrotal testes of the 10ml in size. Varicocele grade 2 was palpated on the left side. The following findings were reported after molecular analysis of androgen receptor gene in these patients. 1. The patient with CAIS had mutation CCT (Arg) 6 CAT (His) in codon 615, exon 3 of the androgen receptor gene. 2. The mother was found to be heterozygotic for the same mutation. 3. No mutation was found in the androgen receptor gene of the patient with female phenotype and male karyotype. She was suggested to have partial Srx reductase deficiency. 4. Sequence analysis of the androgen receptor gene in patient with PAIS revealed a CC to TT transition in codon 865 of exon 7, predicting a Ser to Phe substitution. This mutation has not previously been described. Conclusion. We detected a novel mutation of androgen receptor gene in patient with PAIS.

P3/4 - 050
MODULATION OF TESTICULAR HORMONE SENSITIVE LIPASE (HSL) PROTEIN LEVELS AND ACTIVITY, AND OF TUBULAR AND INTERSTITIAL CHOLESTEROL AND TRIGLYCERIDES (TG) IN RELATION TO SERUM HORMONE PROFILES DURING PUBERTY AND THE ANNUAL SEASONAL REPRODUCTIVE CYCLE IN THE ADULT MINK

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HSL protein levels and activity were measured in seminiferous tubule (STF) an interstitial tissue (ITT) enriched fractions obtained during puberty and the annual reproductive cycle in adult mink. Two HSL immunoreactive bands of 104- and 108-kDa were detected in puberal and adult STf, in addition, a 100-kDa band was detected in STF of adults. In ITF, the 104- and the 108 bands were detected. HSL activity was low in STF and ITF during puberty and peaked with adulthood. During the annual cycle, HSL enzymatic activity followed the spermatogenic activity. Free/esterified cholesterol ratios in STF and ITF followed a profile similar to HSL activity. TG peaked in STF and ITF coincidentally with a decrease in HSL activity during testicular regression. No correlation was found between serum LH and FSH levels and HSL activity in STF and ITF. HSL activity in ITF was negatively correlated with serum PRL levels. Serum testosterone levels were positively correlated with HSL activity in STF and ITF. Hormones may help regulate HSL-dependent free cholesterol levels adequate for the development and maintenance of spermatogenesis. Support NSERC to RMP and MLV.
P3/4 – 051

ISOLATION OF PRIMORDIAL GERM CELLS FROM PIG FOETUSES.


This work was aimed at isolation of primordial germ cells (PGCs) from the foetuses of pigs slaughtered at varying periods of gestation and culturing cells in vitro. We studied the influence of age (days post coitum) of foetuses on isolation of PGCs and reception colonies of stem germ cells, which have embryonic stem cells (ES) morphology. Therefore, we used the gonadal ridges from Day 23, 26, 31 and 33 foetuses in this study to isolate PGCs. Isolation of PGCs was carried out using method developed by us previously on mice. The method includes purification of PGCs from somatic cells based on differential cell adhesion. The selection of PGCs was based upon morphological criteria. The activity of alkaline phosphatase in these cells was used as a specific marker. We studied factors influencing survival PGCs in culture and reception colonies with ES-like morphology. Isolated PGCs were plated on mitotically inactivated feeder layers submitted by two types somatic cells: murin embryonic fibroblasts (STO) and primary porcine Sertoli cells in Dublecco's modified Eagle's medium supplemented with 2 μM glutamin, 15% foetal bovine serum, 0.1 μM 2-mercaptoethanol. We report here that our method allows purification of reasonably pure yields of viable PGCs. It was found that with increase in age of foetus, there was a parallel increase in the number of PGCs. However, colonies of stem germ cells with ES-like were mostly isolated in early foetal development. Using feeder layers we were able to receive 6-8-day colonies of stem germ cells with various morphology, including with ES-like morphology. The feeders tested had an identical effect on the behaviour of plated PGCs.

P3/4 – 052

SECRETION OF LEPTIN FROM ADIPOSE TISSUE OBTAINED FROM DIFFERENT LOCATIONS BEFORE AND AFTER PUBERTY IN THE MALE RAT.

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One component of puberty in the male rat may be an increase in the plasma level of leptin. This idea was supported by the report that the ability of the epididymal fat pad to secrete leptin was enhanced by sexual maturation. The studies reported here were designed to determine if adipose tissue obtained from areas other than the epididymal fat pad also showed an alteration in leptin secretion as a consequence of sexual maturation. Adipose tissue was obtained from immature (n=10) and young adult (n=8) male rats. Subcutaneous fat was obtained from the abdominal region. Epididymal fat was collected from the epididymal fat pad and splenic fat was obtained from the fat pad adhering to the posterior border of the spleen. Tissue from a given animal was minced and incubated for 48 hrs in 2 ml DMEM supplemented with 0.5% fetal calf serum, gentamycin and Penn/Strep with or without the addition of 1 μM dexamethasone plus 50 nM insulin (DEX+). An entire epididymal fat pad obtained from immature animals was used for each treatment while a sample of epididymal fat was sufficient in young adults. Other locations provided sufficient tissue from both ages. Media was assayed for leptin concentration by RIA and the data expressed as pg leptin/mg/48 hrs. The results suggest that epididymal fat is capable of secreting more leptin than subcutaneous fat both prior to and after sexual maturation. However, DEX+ treatment of subcutaneous fat was more effective at increasing leptin secretion from fat obtained from immature animals than from young adults. The data from splenic fat was uninterpretable. Based on these findings, epididymal fat may be the principle source of the increased serum leptin concentrations seen in the male rat after puberty.

P3/4 – 053

CRYPTORCHIDISM AND HYPOSPADIAS RATES IN THE NETHERLANDS.


Introduction: Increasing cryptorchidism and hypospadias trends have been reported, and exposure to endocrine disrupters during fetal life has been suggested as common cause. Published rates are mainly derived from registry systems, which may be inaccurate, as not all cases are assessed and reported, and the denominator is approximated. The rise in hypospadias might be explained by a trend to increasingly report cases of minor severity. The distribution of hypospadias grades is, however, unknown. The aim of our study was to accurately assess the incidence of hypospadias subtypes and cryptorchidism in a complete birth cohort. Methods: From Sept. 1998 to Sept. 2000, 7,292 consecutive male births in Rotterdam were screened for both anomalies. Median age at examination was 35 days (5th and 95th percentiles are 25 and 120). Cases were verified and classified by a urologist. Results: The incidences of cryptorchidism and hypospadias were 1.1 and 0.7%, Of 53 hypospadias cases, 12 were glandular, 15 coronal, and 21 penile, penoscrotal or scrotal. Discussion: Few studies have reported on complete screening for cryptorchidism in the general population. Our incidence of 1.1% is in accordance with comparable studies. The hypospadias rate in Rotterdam was about 4 times the rate given by the European Registration of Congenital Anomalies (EUROCAT). If we exclude glandular cases as EUROCAT does, the rate is still 3 x higher. Only 23% of hypospadias were minor cases. Therefore, the hypothesis that the doubled hypospadias rate reflects an increasing tendency to report minor cases can be refuted. Other explanations, including endocrine disrupters, should be considered.

P3/4 – 054

STERIOGENIC CHARACTERISTICS OF HUMAN LEYDIG CELL PRECURSORS (hLCP) DURING INFANCY.


Human LCP are able to produce testosterone (T) in culture, and initiate differentiation to Leydig cells in response to chronic treatment with hCG. A strong localization of 3βHSD and T was observed by immunocytochemistry on hLCP at successive stages of differentiation toward Leydig cells. This study was performed to evaluate the steroidogenic characteristics of hLCP in culture. Basal, dihydrotestosterone (DHT) and 3α androstadiol (Diol) were determined at the time of maximal steroid production (days 9-11 in culture), percentage of total steroid production was: T: 77.6±11.6; DHT: 19.7±9.6; Diol: 2.7±2.5 (x±SD). No response to hCG (50 ng/ml, hCG CR-127) in 3 hours incubations was observed (Basal: 1.5±0.3; hCG: 1.7±0.1, ng T/μg DNA/ 3 hs, x±SD). Cholera toxin (CT, 50 μg/ml), forskolin (FK, 2.5 μM), dibutyryl cAMP (dbcAMP, 0.1 μM) and hCG (50 ng/ml) plus 3-isobutyl-1-methytykantin (0.125 μM) (hCG-MX) produced the following fold increases in testosterone production: CT: 2.8; FK: 4.2; dbcAMP: 3.6 and hCG-MX: 3.3. In incubations with increasing concentrations of 22 (R) hydroxycholesterol (22OHChol) testosterone production was: Basal: 1.5±0.2; 22OHChol: 0.1 μM: 3.5±0.5; 1μM: 5.7±0.6; 5μM: 12.1±0.9 (ng T/μg DNA/ 3 hs, x±SD). Our results indicate that infantile hLCP produce a small proportion of 5α reduced steroids. In addition, these results suggest that hLCP possess a full complement of steroidogenic enzymes but have not developed the complete intracellular machinery necessary to attain an acute response to hCG.
**P3/4 – 055**

A NOVEL INJECTABLE TESTOSTERONE UNDECANOATE (TU) DOES NOT LEAD TO SUPRAFISIOLOGICAL TESTOSTERONE CONCENTRATIONS IN THE TREATMENT OF MALE HYPOGONADISM

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Testosterone replacement therapy is well established in the treatment of male hypogonadism. Before transdermal formulations became available more recently, i.m. injections of TE were considered as standard. T deficiency is associated with a variety of symptoms: loss of libido, erectile dysfunction, insulin receptor resistance, abdominal obesity, osteoporosis, disturbances of the lipid metabolism, myocardial and circulatory disturbances, impaired well-being and mood. In an open label, randomized, prospective study we compared a novel, long-acting TU formulation (1000 mg three times every 6 weeks, thereafter every 9 weeks) to TE (250 mg every three weeks) in 40 hypogonadal men (T < 5 nmol/L) following a wash-out period of at least 8 weeks. In contrast to the TE group, T levels in patients receiving TU remained within the physiologic range. Results measured in weeks 0 and 30: In both treatment groups, an increase was seen in hormone levels (T: TU 3.9 and 16.3, TE 2.7 and 8.3 nmol/L, p < 0.01 between groups; DHT: TU 3.0 and 10.0, TE 0.4 and 0.5 nmol/L, p < 0.01 between groups; E2: TU 21.5 and 29.6, TE 23.1 and 27.5 pg/ml), hemoglobin (TU: 14.35+/-1.04 and 15.70+/-1.24, TE: 14.73+/-0.81 and 15.90+/-1.05 g/dL), hematocrit (TU: 43.35+/-3.05 and 46.75+/-3.34, TE: 44.35+/-2.21 and 47.81+/-3.04 %), lean body mass (DEXA: TU: 58.5+/-10.3 and 62.6+/-9.2, TE: 59.2+/-9.9 and 62.0+/-8.4 kg), grip strength (right: TU: 40.1+/-10.3 and 44.1+/-10.2, TE: 47.5+/-10.1 and 51.2+/-11.4 kp, left: TU: 37.5+/-10.8 and 41.4+/-10.7, TE: 44.6+/-9.2 and 48.5+/-8.1 kp). Bone mineral density remained unchanged.

**P3/4 – 056**

HEMOGLOBINOPATHIES AND GONADAL AXIS IN MALES: A CROSS-SECTIONAL, MULTI-CENTER, CLINICAL STUDY IN A GREEK POPULATION

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**Background.** Hemoglobinopathies such as thalassemia major (TM) and sickle cell disease (SCD) are characterized by reproductive problems. Alms. (1) to describe the clinical characteristics of patients with hemoglobinopathies regarding gonadal axis, (2) to describe their laboratory profile and (3) to determine prognostic features for gonadal status. Methods. 67 patients with TM and 7 patients with SCD were studied through history, physical examination, spermiograms and gonadotrophin-releasing hormone test.

Results. TM patients were divided into three subgroups. Eugonadal (45%), hypogonadotropic hypogonadism of late onset (21%) and hypogonadotropic hypogonadism of early onset (34%). SCD patients were divided into two groups: Eugonadal (29%) and hypergonadotropic hypogonadism (71%). Current gonadal status of TM patients could not be predicted by means of transfusion or chelation parameters. Bone densitometry in TM patients revealed a bone density reduction of 9.7%/-4.7%, as compared with normal individuals of same sex and age. Conclusions. TM patients can be classified into eugonadal and early or late onset hypogonadotropic hypogonadal whereas SCD patients can be classified into eugonadal and hypergonadotropic hypogonadal. The prevalence of endocrine complications, such as insulin dependent diabetes and hypothyroidism in TM patients is lower than previously recorded.

**P3/4 – 057**

TESTOSTERONE THERAPY INDUCES SOMATIC AND DENSITOMETRIC CHANGES IN MALE HYPOGONADISM


Hypogonadism is increasingly diagnosed in men. Long term effects (LTE) of testosterone replacement (TR) on body composition (BC), bone mineral density (BMD) and total calcium content (TCC) are still controversial. The aim of this study was to quantify the LTE of TR on TCC and BC and its correlation with hormonal and BMD changes. We investigated 15 men, mean age 31.3 years (18-51). 11 primary and 4 idiopathic hypogonadotropic hypogonadism, with serum testosterone (T) <3 ng/ml. Baseline evaluation included: weight, height, body mass index (BMI); T, bioavailable T (BioT) and estradiol (E2) serum determinations; TCC: BMD in spine, trochanter, femoral neck and whole body (SBMD, TBMD, FNBMD, WBMD); percentage of total body fat, abdominal fat and lean mass (%TBF, %AF, %LM) by DEXA. Patients didn’t change their diets or physical activity during treatment. Testosterone enanthate IM, 250 mg twice a month, normalized biochemical parameters at 3 and 6 months. DEXA measurements were repeated at 6 and 12 months. %TBF and %AF decreased under treatment (p<0.001) lean:fat ratio (LFR) and BMD increased (2.06±0.71 vs. 2.85±0.88, p=0.0052 and SBMD p=0.0007, TBMD p=0.0016, FNBMD p=0.0003; TCC p=0.022). TCC correlated with increments in SBMD (r=0.76, p<0.05); T (r=0.78, p<0.05); BioT (r=0.78, p<0.05) and E2 (r=0.62, p<0.05). SBMD also correlated with BioT (r=0.76, p=0.01). The LFR and FNBMD increments correlated significantly (r=0.91, p=0.01). We concluded: TR in male hypogonadism decreases TBF and AF mass with maintenance of BMI and increment in LM. The FNBMD increment could be explained by changes in muscle mass and TCC increments could depend on improvement of SBMD by increments in steroids levels.

**P3/4 – 058**

CIRCADIAN OSCILLATIONS OF MELATONIN (MEL) AND PITUITARY-GONADAL AXIS HORMONES IN MALES WITH CHRONIC ACTIVE HEPATITIS B (CAH) AND LIVER CIRRHOSIS (LC):

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Normal activity of the pituitary-gonadal axis depends, amongst other factors, on the functional state of the liver. The pathogenesis of hypogonadism in liver diseases is not completely understood. The liver plays an important role in the intermediate metabolism of numerous substances involved in hormonal action. Most data point to the influence of the pineal gland on the sexual system. It is possible that rhythm disturbances MEL diurnal in liver disease play a pathogenic role in causing the altered sexual activity. 24-hours MEL, gonadotropins (LH and FSH) and free testosterone (FT) secretion in patients with LC and CAH were assessed. Not many reports have been written on that so far and studies concerning chronobiological aspect of this problem are rare. Studies were performed in 15 patients with CAH (mean age 33.1) and in 15 with LC (mean age 44.5 years). the control group consisted of 15 males (mean age 38.5 years). Blood was collected at 8.00 AM, 11.00 AM, 2.00 PM, 5.00 PM, 8.00 PM, 11.00 PM, 2.00 AM, 5.00 AM. Hormones were assessed by RIA. Assesment of chronobiological parameters of particular groups was done with the use of the cosinor method by Halberg. In the control group the existence of daily MEL rhythm with acrophase at night was observed, this is consistent with reports in the literature received up to now. In groups of patients with LC and CAH B loss of MEL circadian rhythmicity in blood, as well significant increase of daily secretion of this hormone in relation to control group was observed. Decrease of daily FT secretion was noted in LC group.
P3/4 – 059
ANDROPAUSE AND ORAL TESTOSTERONE REPLACEMENT THERAPY. DESCRIPTION OF OUR EXPERIENCE IN 76 PATIENTS WITH PRIMITIVE HYPOGONADISM.
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INTRODUCTION: Appropriate designation of Andropause is Androgen Decline in the Aging male (ADAM) and consists of physical, psychological and sexual disturbances due to Testosterone (T) level decline. During male ageing T levels decline, on average, at a rate of 1% yearly after the age of 40 years. About 20% of men aged 60-80 years have levels below the lower normal threshold. In this study, we consider the epidemiological aspects and clinical manifestations of ADAM, and provide treatment and monitoring of a group of patients, to verify the efficacy of oral T replacement therapy.

MATERIALS AND METHODS: Over the last two years 76 of our patients (pts), aged 45-72 years old, were selected. All 76 patients came to the clinic complaining of erectile dysfunction and of decrease of libido. Of these, 14 patients were affected by diabetes, 25 presented essential hypertension and 8 were affected by cardiovascular disease. All of them presented a primitive hypogonadism. Patients were submitted to the following tests: physical exam, sex hormones and Prostate Specific Antigen (PSA) serum level determination and trans-rectal ultra-sonography to exclude prostate malignancy. The International Index for Erectile Dysfunction (IIEF) and the Mini Mental Statement Examination (MMSE) questionnaire were administered. All patients received oral Mesterolone in dose of 50 to 150 mg daily, on the basis of the T serum level, for at least 6 months. Pre-treatment tests were repeated at 3 months and at the end of the therapy.

RESULTS: the mean average of total score of MMSE improved from 21 to 27.

P3/4 – 060
CHROMOSOME ANALYSIS IN BROTHER CASES OF KALLMANN’S SYNDROME, USE OF FLUORESCENCE IN SITU HYBRIDIZATION, COMPARATIVE GENOMIC HYBRIDIZATION, AND MULTICOLOR SPECTRAL KARYOTYPING.
T.Kobayashi*, Y.Ioe, Y.Tokunaga, M.Morioka, H.Tanaka, Department of Urology, Kawasaki Medical School, Kurashiki, Japan.

Material: The patients were two brothers, the elder one being 16 years old (case 1) and the younger one being 15 years old (case 2) who did not present secondary sexual characters. Central anosmia was found both cases. Their past history revealed operation for bilateral undescended testes. Their appearance corresponded to grade 1 of the classification of Tanner and genital stage 1. The plasma levels of LH, FSH and testosterone were low and their circadian rhythm was not recognized. The LH-RH loading test, the continuous LH-RH loading test and the hCG loading test showed a normal response in both cases. Their chromosome was normal to the karyotype analysis (G-banding stain). The patient’s lymphocytes were cultivated in a CO2 incubator during 3 days, followed by hypotonic processing, slide chromosome displa and steam fixation. Fluorescence in situ hybridization (FISH) was performed with a DNA probe to the KAL gene (Xp22.3) and to the centromere (Xp11.1-q11.1). FISH protocol consisted of: 1. Preparing the slides, 2. Preparing the probe mixture, 3. Hybridization of the probe to the slides, 4. Washing the slides after hybridization, 5. Visualization and analysis of the slide. Comparative genomic hybridization (CGH) and multicolor spectral karyotyping (M-FISH) were performed in both cases. CGH allowed to determine the loss and gain of chromosome fragment and M-FISH was utilized for analyzing the new dislocation.

P3/4 – 061
INHIBIN B AND SPERMATOGENESIS IN HYPOGONADOTROPIC HYPOGONADISM TREATED WITH HCG AND RECOMBINANT FSH
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Induction of spermatogenesis in males with hypogonadotropic hypogonadism (HH) requires the administration either of LH plus FSH or GnRH. Data on recombinant FSH (r-hFSH) therapy in males with HH are very limited. Aim of this study was to assess the efficacy of low doses of r-hFSH (Gonal-F, Serono, Italy) compared to low doses of highly purified urinary FSH (FSH-HP, Metrotin HP, Serono), in combination with hCG, to induce spermatogenesis and normalize inhibin B levels in patients with congenital HH. Eighteen men with congenital hypogonadotropic hypogonadism (19-32 yr old) were enrolled. All patients received hCG (Profasi HP, Serono) for 6-12 mos (pretreatment); then 75 IU of r-hFSH (group 1, n. 10) or FSH-HP (group 2, n. 8) 2 times weekly in combination with hCG. Semen analysis and hormonal evaluation was performed before treatment and at 3-months intervals for 21 mos. Results: Inhibin B reached maximal levels not significantly different between two groups (186 + 22.5 vs. 198.7 ± 42.1 pg/ml). A sperm concentration > 1 mil/ml was found in 50% of cases in group 1, and in the 85.7% in group 2 after 6 mos FSH administration. Maximal mean sperm concentration was 14.4 ± 5.2 in group 1 and 29.0 ± 3.0 x10^6/ml (±SE) in group 2 (p<0.03). Testicular volume resulted no significantly different. Conclusions: We demonstrated that FSH and FSH-HP are efficient in stimulating tubular function at lower doses (75 IU two times weekly) than reported (150 IU of FSH three times weekly), with a reduction of costs and a good compliance of patients to the therapy. Moreover, using the same doses of r-hFSH and FSH-HP, we found better results in the r-hFSH group regard to sperm output.

P3/4 – 062
THE REPRODUCTIVE AND SEXUAL FUNCTIONS IN UREMIC MALES
Abdalla M. Attia and Samea H. Kandil*
Andrology and Clinical Pathology Depts., Minufia University.

Hypogonadism is a frequent manifestation of uremia. Its exact etiopathology and effective therapy are – until now – unknown.

This study included 25 uremic males undergoing regular hemodialysis and aging 20-50 years. They were subjected to: measurement of the testicular size and consistency, estimation of their sexual function (answering a designed questionnaire) as well as estimation of their serum levels of FSH, LH, Free T (FT), PRL, zinc (Zn) and cadmium (Cd). Fifteen healthy normal-age matching-subjects were selected as a control group for comparison.

The results showed that; uremic patients show significant reduction of testicular size with softness of the consistency (52%), loss of libido and significant erectile dysfunction (E0-E2) in 76% . Elevation of FSH, LH, PRL, and Cd, reduction of Zn and insignificant lowering of FT levels.

We think that; the elevated PRL and Cd and the reduced Zn are some- if not the major- causes of the hypogonadism reported in the uremic males.
P3/4 – 063
SUCCESSFUL TREATMENT OF MALE HYPOGONADISM WITH TESTOSTERONE UNDECANOATE (TU) INJECTIONS IN EXTENDED INTERVALS OF 12 WEEKS.
S. v. Eckardstein, D. Büchter, E. Nieszlag Institute of Reproductive Medicine of the University, Münster, Germany
Currently available testosterone (T) preparations for substitution therapy of male hypogonadism are short acting and require injections in 2 to 3 weeks intervals. In a previous study we demonstrated that injections of LIU every 6 weeks resulted in satisfactory substitution. We now investigated further extension of injection intervals. In an open non randomised clinical study 7 hypogonadal men received first 4 injections of 1000 mg TU solved in 4 mL castor oil every six weeks. Treatment was then continued with another 9 injections in gradually increasing intervals and another 5 injections given every 12 weeks. Wellbeing, sexual activity, serum hormone levels, clinical chemistry, prostate volume and PSA were monitored. Following the 9th injection T was measured weekly for 12 weeks to establish a kinetic profile. Patients were clinically well adjusted throughout the study. T, DHT and estradiol levels prior to the next injection were mostly within the normal range and showed a tendency to decrease with increasing injection intervals. Body weight, hemoglobin, serum lipids, PSA and prostate volume did not change significantly compared to the six week injection interval. PSA levels were always within the normal limit. Maximal T-levels during steady state kinetics were measured after one weeks with 31.7 + 11.6 nmol/L (mean + SD). Prior to the last injection mean T concentrations were 13.4 + 4.5. Compared to conventional T injection treatments of TU in intervals of up to 3 months offer an excellent modality for substitution therapy of male hypogonadism.

P3/4 – 065
COMPARISONS BETWEEN COUPLES WITH DIFFERENT SMOKING HABITS: MALE AND FEMALE SEXUAL BEHAVIOR.
13PM Zavos, 12CN Zarmakoupis, 1J Correa, 1K Kaskar, 1PN Zarmakoupis-Zavos, 1Andrology Institute of America, Lexington, Kentucky, USA and 1The Kentucky Center for Reproductive Medicine and IVF, Lexington, KY, USA.
Objective: The current study was to assess any effects of smoking on sexual frequency and satisfaction in couples that smoke and those that do not smoke (NS). Also among the group that smoked, the sexual behavior was compared between those where only the male smoked (SM1) and both male and female smoked (SM2).
Materials and Methods: Each couple (n=393) that participated in this study filled out a questionnaire pertaining to their clinical profile and sexual history. The information included: ages of husband and wife, number of years married and years trying to conceive, sexual frequency per month and sexual satisfaction/rating (0=poor; 10=extremely satisfactory). They were also asked to reveal their smoking habits, (number of years of smoking and number of cigarettes smoked per day). Only those patients that smoked 30 cigarettes/day or more were considered in the study.
Results: The results obtained in this study are shown in the table below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (ys)</th>
<th>Age Married (ys)</th>
<th>Men Manic to Conc. (ys)</th>
<th>Cig. smoke (per day)</th>
<th>Freq (times)</th>
<th>Status (0-40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>32 + 2.7</td>
<td>29.5 + 6.7</td>
<td>6.2 + 0.8</td>
<td>2.6 + 0.4</td>
<td>0.0 + 0.0</td>
<td>1.1 + 1.6</td>
</tr>
<tr>
<td>SM1</td>
<td>32.8 + 2.3</td>
<td>29.3 + 6.7</td>
<td>3.1 + 0.5</td>
<td>3.1 + 0.5</td>
<td>2.6 + 0.4</td>
<td>1.1 + 1.6</td>
</tr>
<tr>
<td>SM2</td>
<td>30.3 + 1.4</td>
<td>27.4 + 4.0</td>
<td>6.1 + 1.6</td>
<td>3.3 + 0.0</td>
<td>2.6 + 2.7</td>
<td>3.1 + 2.5</td>
</tr>
</tbody>
</table>

The results showed that smokers males experienced higher sexual frequency and sexual satisfaction than couples where only the male or both partners smoked. We also showed that the male seems to be more involved in causing these deficiencies as no additive detrimental effects were noted when the female also smoked. It could also be possible that the lack of additive effects is due to that, when one partner in the couple smoked, the cigarette effect was distributed to both via "second-hand smoking". We established that smoking adversely affects sexual behavior and anyone who smokes and suffers from infertility should be advised to stop.

P3/4 – 066
PREDISPOSING FACTORS FOR THE TRAUMATIC PENILE RUPTURE.
AF De Rose, M Giglio*, and G Carignanii* Department of Urology, Genoa, Italy.
INTRODUCTION: Fracture of the corpora cavernosa represents an uncommon but certainly under-reported event. It is caused by blunt trauma to the erect penis resulting in tearing of the tunica albuginea. The aim of our paper is to investigate the physiopathology of the rupture of corpora cavernosa, by means of a histological study of the tunica albuginea. MATERIAL & METHODS: During the last year we observed six patients with traumatic rupture of the penis. All injuries occurred during sexual intercourse and were due to the violent impact of the erect penis against the partner's perineum. Multiple samples of the tunica albuginea were taken at least two cm far from the tear in all the six patients during reparative surgery. We took other samples of tunica albuginea in other seven patients who were subjected to penile surgery for other reasons. All patients did not present systemic diseases and had not undergone any previous radiation or steroid therapy. RESULTS: At light microscope examination we identified large areas of fibrosclerosis of the tunica albuginea in five of the six patients (83%) observed for penile rupture. Flogistic cellular infiltrations composed of lymphocytes or histiocytes were present in three of these. Only one patient (17%) with traumatic penile rupture presented a completely healthy tunica albuginea free from histological anomalies. None of the control specimens revealed either fibrosclerosis or inflammatory cellular infiltrations. CONCLUSIONS: The presence of histological structural anomalies in the tunica albuginea could represent a weakening factor of the corpora cavernosa and a predisposing factor for the traumatic rupture of the penis. Therefore, these sick tunicae albugineae could be subjected to rupture even at intracavernous pressure greatly inferior to ones necessary to determine any tear.
Abstracts – Poster Session 3/4

P3/4 – 067
ERECTILE FUNCTION OF BICYCLING POLICE OFFICERS.

In response to complaints of groin numbness in a bicycling police unit, a health assessment study was conducted. Seventeen bicycling officers were compared to 5 non-biking men. The biking officers rode an average of 5.4 hours per day and 91% indicated they experienced groin numbness on occasion. Each man wore the Rigiscan Rigidity Assessment System for one normal sleep session. Seating pressure measurements were also taken between the cyclist and the bicycle seat. The percent of a sleep session that recorded an erectile event was significantly lower in the bikers compared to the non-bikers (bikers 27.1%; non-bikers 42.8%; p=0.008). This duration percent is negatively correlated with average hours a day the officer rides his bike (r=-0.41; p=0.05), the number days a week he rides (r=-0.55; p=0.009), and the average pressure exerted on the nose of the bike seat (r=-0.39; p=0.08). The other measures of erectile quality (TAU and RAU of both the base and tip of the penis) were lower in the bikers, but did not reach statistical significance. The number of hours the officer rode the day of the Rigiscan assessment was negatively correlated with the penis tip tumescence (r=-0.46; p=0.04), tip RAU (r=-0.41; p=0.04), and tip TAU (r=-0.45; 0.04). These data suggest that prolonged bicycle riding may have negative effects on nocturnal erectile function.

P3/4 – 068
THE EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON SEXUAL FUNCTION. Manoj Monga, Mehdi Kamarche*, UCSD Medical Center, San Diego, CA

Outcome analysis of testosterone supplementation for erectile dysfunction (ED) is limited. We evaluated long-term efficacy using standardized questionnaires and compared the results with different testosterone delivery systems.

Forty-four men received depot-testosterone (DPT, 400 mg IM q2-3 wks) Testoderm scrotal patches (TSD, 4-6mg/24 hr) or Testoderm-RTS non-scrotal patches (TTS, 5mg/24 hr) at the VAHCS San Diego for ED between 1/96-3/98. The ED inventory of treatment satisfaction (EDITS) and sexual health inventory (SHIM) questionnaires were administered. Global questions were asked regarding improved libido, energy, erections. Co-morbidities included smoking (34%), diabetes (34%), cardiovascular disease (64%), hypertension (54%). Average duration of ED was 5.8 years. Mean pre-treatment serum testosterone was 132.3 ng/dL (normal 180-650). Pre-treatment ED was characterized as mild (9%), moderate (26%) or severe (65%).

<table>
<thead>
<tr>
<th></th>
<th>DPT (n=15)</th>
<th>TSD (n=22)</th>
<th>TTS (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59</td>
<td>56</td>
<td>59</td>
</tr>
<tr>
<td>Months follow-up</td>
<td>119</td>
<td>38</td>
<td>59</td>
</tr>
<tr>
<td>Improved libido</td>
<td>73%</td>
<td>38%</td>
<td>86%</td>
</tr>
<tr>
<td>Improved energy</td>
<td>53%</td>
<td>38%</td>
<td>86%</td>
</tr>
<tr>
<td>Improved erections</td>
<td>40%</td>
<td>31%</td>
<td>57%</td>
</tr>
<tr>
<td>Average EDITS</td>
<td>2.79</td>
<td>4.11</td>
<td>2.06</td>
</tr>
<tr>
<td>Average SHIM score</td>
<td>2.42</td>
<td>4.28</td>
<td>2.14</td>
</tr>
<tr>
<td>Discontinuation rate</td>
<td>13%</td>
<td>73%</td>
<td>0%</td>
</tr>
</tbody>
</table>

86% of patients receiving Testoderm-RTS reported an erect on satisfactory for intercourse (SHIM question 5) all or most of the time.
Testosterone supplementation improves quality of erections and level of libido and energy in the majority of patients. Treatment delivery systems impact on the success of therapy.

P3/4 – 069
DEHYDROEPIANDROSTERONE SULPHATE (DHEAS) AND THE LIPID STATUS IN THE ERECTILE DYSFUNCTION
Ph. Kumanov, A. Tomaova
Clinical Center of Endocrinology and Gerontology, Medical University, Sofia, Bulgaria

The results of the Massachusetts Male Aging Study (MMAS) on male subjects 40 to 70 years of age showed that only dehydroepiandrosterone sulphate (DHEAS) out of 17 hormones had a strong correlation to erectile dysfunction and that the probability of this disorder varied inversely with high-density lipoprotein cholesterol (HDL-CH). The current study was undertaken in an attempt to acquire a better insight into the role of DHEAS, testosterone and lipids on age related deterioration in erectile function. Twenty-five males (9 under 40 years of age and 16 over 40) with erectile dysfunction were investigated. Ten healthy subjects (aged 18-77 years) served as a control group. We evaluated the serum levels of DHEAS and testosterone by RIA and lipids by enzymatic colorimetric test.

Plasma levels of DHEAS were significantly lower in patients over 40 years of age (4.63±3.16 μmol/l, X±SD) as compared to the younger group (0.33±4.69 μmol/l), p<0.001, the levels in the controls being 9.66±1.88 μmol/l. DHEAS showed an inverse correlation with age (r=-0.661, p<0.001) and positive with testosterone (r=0.565, p<0.01). There was no statistically significant difference between the two groups of patients in lipid data, which were in the reference range, including HDL-CH.

The current study is cross sectional, as is MMAS, and it shows that lipids and DHEAS do not have a crucial influence on erectile function. More data, especially from longitudinal studies is needed to elucidate the importance of them for sexual disorders.

P3/4 – 070
SEXUAL DYSFUNCTION IN COMBAT VETERANS WITH POST-TRAUMATIC STRESS DISORDER.
Daniel I. Cosgrove*, Jonathan E. Berrie*, Zachary Gordon*, Murray Stein*, Manoj Monga, UCSD Medical Center, San Diego, CA

Recent studies suggest increased rates of sexual problems among patients with post-traumatic stress disorder (PTSD). We evaluated the incidence and clinical correlates of sexual dysfunction among combat veterans with PTSD. Male Vietnam era combat veterans who receive treatment for PTSD at the Veterans Affairs Medical Center PTSD clinic, and an age-matched control group of Vietnam era combat veterans not being treated for PTSD completed questionnaires at a clinic visit. The packets included a demographic and health questionnaire, the World Health Organization (WHO) disability survey, and the International Index of Erectile Function (IIEF). All five IIEF domains were scored for each responder. Combat exposure and PTSD status were assessed by two self-report measures, the Posttraumatic Stress Disorder Checklist and the Combat Exposure Scale.

Completed questionnaires were returned by 58 of 65 patients (40 with PTSD and 18 controls). Mean age was 51. Compared to controls, patients with PTSD reported poor orgasmic function (1.61 vs 3.33, p=0.08) and overall sexual satisfaction (4.11 vs 6.13, p=0.02). No statistical significance in erectile function (9.28 vs 9.20, p=0.87), sexual desire (3.94 vs 4.63, p=0.45), and intercourse satisfaction (3.11 vs 3.65, p=0.70) was noted. Combat veterans receiving outpatient treatment for PTSD appear to suffer more sexual dysfunction compared to an age-matched control group of Vietnam combat veterans without PTSD. Psychosocial explanations for these findings include blunting of affect seen in patients with PTSD, relapse of traumatic memories induced by sexual activity and access to fewer partners. Co-existent medical and psychological problems can further contribute to erectile problems in this population.
P3/4 – 071
CLITORAL NITRIC OXIDE SYNTHASE ISOFORMS IN A SWINE CLITORAL CELL CULTURE MODEL.
Mahadevan Rajasekaran, Valeria Pagnon*, Manaj Monga, UCSD Medical Center, San Diego, CA.
The mechanisms regulating female clitoral erectile function are not well-defined. Clitoral smooth muscle is the primary cellular component of female erectile tissue, and nitric oxide (NO) has been proposed as the main mediator of clitoral erectile function. Clitoral NO synthase (NOS) isoforms have not been characterized. We developed a swine clitoral cell culture and characterized the ex vivo expression of NOS isoforms in these cells. Swine clitoral tissue obtained at necropsy was placed into culture flasks containing growth medium (Dulbecco’s MEM + 20% fetal BSA) and explant incubated at 37° C. Swine clitoral smooth muscle cells (SCSMC) were characterized for the presence of smooth muscle cell specific markers (actin, myosin), endothelial cell-specific marker (von Willebrand’s factor [vWF]), and NOS by indirect immunofluorescence assay. SCSMC were grown on glass chamber slides (70-80% confluence) and fixed with 4% para formaldehyde for 30 minutes. Cells were incubated overnight at 4°C with specific monoclonal antibodies for smooth muscle specific actin (1:100), myosin (1:100), vWF (1:100) and endothelial NOS (eNOS) (1:200) then treated with appropriate secondary antibody for two hours. Slides were mounted and observed under a Leitz fluorescent microscope. SCSMC exhibited a characteristic hill-and-valley appearance in culture. The immunofluorescence assay showed a positive label for smooth muscle specific markers (actin, myosin), and were negative for endothelial cell-specific marker [vWF]. Cells were positive for eNOS which predominantly localized to the cell membrane. SCSM in culture is a viable model for ex vivo evaluations. eNOS appears to be the predominant isoform involved in the regulation of female erectile function. Supported in part by an AFUD/AUA summer scholarship to VP.

P3/4 – 072
PERMANENT PROLARGEN OF THE PENIS “ELSEWEIFI I TECHNIQUE”
Aref El-Seeifi * Center of Urology in Bogenhaus, Potsdamer Chaussee 80, 14129 Berlin, Germany.
Lengthening of the penis, which is an important add to the confidence of some men, is remarkable only in tumescent state. It is achieved through the incision of the suspensory ligament to advance the base of the corpora anto-caudally through an infrapubic v-y plastic. The drawbacks are: presence of hair at the base of the shaft, scarring and retraction of the skin leading to disfigurement, reattachment leading to recurrence or even shortening, scrotalisation of the penis and destabilisation of the erected penis. I present the following new technique: a transverse infrapubic skin incision is done to be closed longitudinally. The suspensory ligament is severed. Two sheets of polypropylene/ polygactin 910 (VYPRO, II) network (Ethicon, Germany) are fixed opposite each to the pubic rami and to the base of the shaft dorsally. The space in between is reduced to minimum. The penoscrotal skin folds are excised longitudinally. 40 Patients (35-64 years old) have been operated as outpatients within 120 minutes since 12 months. An individual gain of length between 3-5 cm has been achieved. The overall complications rate was 17.5%. These were prolonged serous oozing over the miniredon for 1 week in 2 patients (2.5%). The second but most frequent complication is the skin dehiscence in the centre of the wound (3-4 mm long) present in 5 patients (12.5%). Elseweifi I technique for penile prolongation provides permanent results and minor complications.

P3/4 – 073
PERMANENT THICKENING OF THE PENIS “ELSEWEIFI II TECHNIQUE”
Aref El-Seeifi * Center of Urology in Bogenhaus, Potsdamer Chaussee 80, D-14129 Berlin, Germany.
Thickening of the phallus to build self confidence of some men is performed through the injection of biogel, (private lab, Kiev, Russia), autologus fat, Vaseline, or the implantation of autologus derma fat grafts, pedicle fat flap, AlloDerm,(Ilfecell, Branchburg, USA) or Permacol, (tissue science laboratories, Aldershot, England). These procedure resulted in infection, deformation, absorption, calcification, limited thickening, recurrences and high expenses. I report on a new technique for thickening through the implantation of polypropylene/ polygactin 910 (VYPRO, II) network (Ethicon, Germany) bilateral and dorsal to the corpora cavernosa. The operation is performed on outpatient basis under local infiltration of anaesthesia within 40 minutes. Incisions are done in the sulcus coronarius and under the per-scrotal folds laterally, respectively. Subcutaneous tunnels are developed. Folded VYPRO, II is implanted. The wounds are closed and compression is applied. 37 patients (26-64 years old) have been operated upon in on year. The circumference of the shaft have been symptomically increased up to 30%. The overall complications rate was 8.1%. Of these, one patient suffered temporary skin oedema (2,7%), another suffered flatttening of the shaft which was corrected by dorsal implantation of the material (2,7%) and the third a slight rotation of the shaft (2,7%). Elseweifi II technique provides permanent results with minor complications.

P3/4 – 074
CARBON DIOXID (CO2) LASER THERAPY OF PEYRONIE’S DISEASE
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INTRODUCTION AND OBJECTIVES: Authors review the combination therapy of Peyronie’s disease (PD) using CO2 laser vapourisation of nodules and plaques with the contralateral Nesbit type plication stitch. Results and side effects are discussed.
MATERIALS AND METHODS: Between 1985 and 1999 (15 years interval) 54 patients suffering from PD was treated with CO2 laser vapourisation (43 patients with CO2 laser only, 11 patients with CO2 laser combined with contralateral Nesbit type plication stitch). Mean age 56.2 years. The indication of therapy included unsuccessful medical therapy or observation lasted more than a year and curvature more than 30 degree hindering sexual intercourse. The plaque was in dorsal position in 49 cases, ventral in 3 cases and bilateral in 2 cases. Multiple plaques were found in 18 cases. Contours of the plaque were well-defined in 41 cases, while ill-defined nodules, propagating under the glans or the septum were seen in 13 cases. Firstly biopsy, then the vapourisation of the nodule was carried out with CO2 laser using power of 5-10 Watt and continuous operation method. In 11 cases when intraoperative artifical erection was inappropriate, we made a contralateral sided Nesbit plication stitch.
RESULTS: Curvature was tested with ICI after 2 months, sexual function was evaluated after 3 months, pain was evaluated after 6 months. All patients reported of improvement compared to preoperative conditions. Straight penis without angulation was reported in 46 cases, in 8 cases the curvature was on the same direction as the original but decreased. Erection and sexual intercourse was normal in 52 patients, but two patients had erection only with the aid of ICI.
**P3/4 – 075**

SUCCESSFUL MANAGEMENT OF HIGH-FLOW PRIAPISM WITH SUPERSELECTIVE EMBOLISATION

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INTRODUCTION AND OBJECTIVES: The authors present the diagnostic methods and successful therapy of a case of a so-called non-ischaeamic high-flow priapism which developed after a blunt perineal trauma and lasted 8 days.

MATERIAL AND METHODS: A 44 years old male patient suffered from priapism which developed 8 days after a blunt perineal trauma. Color Doppler ultrasonography was performed after admission, following surgery and embolisation and during follow up. A super-selective angiography of the pudendal arteries was performed then the left cavemosal artery was embolised with a mixture of autologous blood clot and absorbable gelatine sponge (spongostan).

RESULTS: Before the admission to our department in another institution a function of the cavemosal body was performed and alpha-adrenergic agonist drugs were administered. The color Doppler ultrasonography (CDUS) that was carried out on our institution showed signs of high flow priapism by measuring 0.29 m/s and 0.4 m/s peak systolic velocity (PSV) in the right and left cavemosal artery, respectively. At first a corpora-cavemosal shunt was prepared, after this the rigidity of the penis diminished but the painless erection recurred soon after surgery. Thereafter was the angiography performed which proved a left-sided arterio-cavemosal shunt. After the embolisation the spontaneous teneuscence of the penis markedly decreased. The CDUS study carried out immediately after the intervention showed a PSV of 0.17 m/s and 0.24 m/s on the right and left side, respectively. By follow-up studies the PSV values decreased to 0.10 - 0.15 m/s. The normal erectile function returned 6 months later.

**P3/4 – 076**

PECULIARITIES OF TREATMENT OF PATIENTS SUFFERING FROM A MIXED COUPLATIVE DYSFUNCTION WITH A LEADING INTERO- RECEPTIVE SYNDROME


The purpose of our research is to develop a treatment scheme for patients suffering from a mixed copulative dysfunction with a leading interoreceptive syndrome. 84 patients with a mixed copulative dysfunction have been examined, out of which 43 patients had trichomonad and 41 patients had bacterial urethra-prostate inflammation. We have developed an individualized scheme of treating patients suffering mixed copulative dysfunction. During the first stage antibacterial therapy was carried out according to the results of antibiotics test taking into account the pathogen type and availability of microbe associations. Treatment complex consisted of a diet containing vitamins A, B, C, E increasing desintoxication function of the liver (application of a hot-water bottle on the liver area after taking some sugar, special therapeutic physical exercises), finger massage of the prostate gland and spermatic vesicles along with hydro massage, electric treatment (Sherbakov's bromine galvanic collar, rectal phonophoresis with 5% dymexide solution, 1% solution of orsasole along with antibiotics), hydro procedures, diuretic medicines. In case of a distinct intoxication inital, splenin were prescribed; to increase sympathetic tonus isadrin, thyreodin; to reduce sympathetic tonus methylpina, dopget; in case of an increased parasympathetic tonus atropine, scopolaminate, etc. In case of increased excitability of both links of autonomic nervous system platifin, spasmylinit, dimedrol were prescribed. Electro stimulation with sinustodial and modulated currents was applied rectally and endouretheterally, injections of bio-stimulating medicines (placenta dregde, aleso, torfot), hormone therapy.

**P3/4 – 077**

PRACTICAL TREATMENT WITH V E P DEVICE

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There are no so many pleasures in this world that man are giving so credit as sexual pleasure. Scientist are making great effort to discover right medicament for demaged man sexual function. Our contribution in solving this problem is practical treatment with method of vacuum erection of penis (VEP) device. This device is attested at Physiological Institute of Medical Faculty in Belgrade, and is highly graded. To prove clinical objectiveness, tests were done in France, at the Sexology Clinic, under supervision from well known Profesor Pjer Lavozaj. Success rate was 80% of tested patients. From 10 000 patients, we have randomly selected 850 patients that are using this device. It was used according to the instructions, no side effects, nor any complaints were reported. Only 2 patients with chronic diabetes mellitus from this group did not have complete erection of penis. Youngest patient was 24 years old, and the oldest was 88. years old. Some minor complication, that we have received reports about, from other colleagues, we are explaining as results of false using, not following the instructions completely. We are totally convinced that VEP device is capable of making erections to almost any man, expt those with circulatory problems.

**P3/4 – 078**

SAVABO SPECIAL - IMPROVEMENT OF THE SEXUAL REFLEX


Dynamics of modern way of life, psychological pressure, and numbered stress factors, have bad influence on the sexual receptors, so some man are afraid to approach their sexual partners. Sexual centers in the brain and thoracic part of the spinal cord are under direct mind control, so all methods of stimulating sex reflex (except VEP devecze) are with low efficacy. Authors have discovered tincture that is named SAVABO SPECIAL. It consists of plant extracts from five domestic plants. This tincture was examined at the Medical Faculty in Belgrade - Institute for Physiology, Department of Pharmacy of Serbia and is still being examined at the Urology Clinic at the Medical Faculty in Novi Sad. Authors are presenting their first experience with 472 man, SAVABO SPECIAL treatment. First provoked morning erection we had second day of in taking this tincture, the most common timing (52%), was fifth day of intake, and the latest was on the fourteenth day. Those that did not react at all was 20%. In the further research we found out that most of those had sclerotic blood vessels, psychiatric diseases and were using heavy doses medications that have influence on the sexual reflex. During the research we found out that this tincture has good effect on the gastric symptoms, and calming effect on the nervous patients. Substances from the SAVABO SPECIAL are harmess and have no date of expire. Together with sexual foreplay, this tincture is helping to reach full erection and capability to copulate.
P3/4 – 079

PENILE PROSTHESIS REIMPLANTATION. R Wang, VA Cancellaro*, J Renehan*, K Lennox*, and RW Lewis, Medical College of Georgia, Augusta, GA.

Introduction: Reimplantation of a penile prosthesis can be a challenge even for experienced urologists. We evaluated the success and techniques involved in reimplantation of penile prostheses.

Materials and Methods: We reviewed the charts of patients who underwent reimplantation of penile prostheses from 9/1995 to 8/2000. The reason for reimplantation, surgical approach, cavernosal dilation techniques, and type of prosthesis as well as complications were assessed.

Results: 58% of reimplantation surgeries were performed to replace the malfunctioned prostheses. 18% of patients had reimplantation because the previous prostheses were removed due to infection. 12% of reimplantations were performed to correct malpositioned prostheses. 12% of patients had reimplantation because their previous prostheses were removed due to urethral erosion. Reimplantation surgeries were performed in 53% of patients through infrapubic incision; 18% by penile scrotal incision; 18% with circumcision-like incision; 5.9% by combination of infrapubic and penile scrotal incisions; and 5.9% with combination of infrapubic and circumcision-like incisions. Multiple small corporotomies, combination use of scissors and dilators or bivalving of corpora were used in 50% of patients with penile fibrosis. Almost all currently available prostheses were used for reimplantation with 88% being inflatable. The mean follow up was 30 month with overall functional prostheses in place in 95% of patients. 13% of patients had reimplant infection with successful third reimplantation.

Conclusion: Penile reimplantation can be successfully performed in the majority of patients with minimal complications.

P3/4 – 080

WHAT DOES POOR RESPONSE TO INTRACAVERNOSAL INJECTION REALLY INDICATE?
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OBJECTIVES: Intracavernosal injection(IC) may be used in a diagnostic capacity to differentiate between the various etiologies of erectile dysfunction(ED). We studied which ED etiology is best predicted by patient poor response to IC test. MATERIALS & METHODS: 256 ED patients evaluated at the Andrology Clinic at Mansoura University Hospital,Egypt, between 1996 and 1999. After a standard ED evaluation and after applying exclusion criteria from the study, 122 remaining patients received a test dose of IC with Trimix(papaverine, phentolamine, prostaglandin-E1). followed by evaluation with nocturnal penile tumescence(NPT)testing, penile blood flow studies(PBF), and redosing pharmacocavernosometry(RPC). Results of these adjunctive tests were compared to outcome of the 'Trimix' ICI test by quantitative analyses. RESULTS: 87 patients (average age 46.8 years) failed to respond to the ICI test. 21% of ICI poor responders had normal Rigsan data, 45% had normal peak systolic velocity(PSV), and 30% had normal flow to maintain erection(MF). Receiver operator characteristic curve (ROC) area to differentiate poor from good responders was 0.86 for MF and 0.69 for PSV. CONCLUSIONS: Based on Rigsan data, the ICI test will lead to an overdiagnosis of organic ED in 21% of patients. Using currently accepted Doppler criteria, 45% of ICI poor responders will not have objective evidence of arterial insufficiency. Based on the high association of abnormal RFC findings in ICI poor responders and its corresponding ROC values, we conclude that venous insufficiency is strongly associated with failure to respond to ICI test.

P3/4 – 081

CORRELATION OF OFFICE INJECTION TESTING (OIT) WITH HEMODYNAMIC EVALUATION: PROSPECTIVELY PERFORMED STUDY.
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INTRODUCTION AND OBJECTIVES: OIT predicts whether a patient will respond to self-injection therapy. It is controversial whether OIT which lacks standardization of techniques, can predict hemodynamic testing outcome. This study was performed to correlate OIT utilizing a standardized procedure with re-dosing hemodynamic evaluation. METHODS: 42 impotent men for at least 6 months (18-75 years) signed an informed consent. OIT was performed using a single 10 mg PGE1 injection. Serial evaluations involving physician-assessed erection grade (1-4) and penile buckling force determination were performed at 10-minute intervals over 30 minutes. A positive test was defined as a grade 4 (fully rigid, fully tumescent), non-buckling erection to 1.0-kg axial load on all three evaluations. All patients then underwent duplex and dynamic infusion cavernosometry/cavernography (DICC) using a previously outlined redosing schedule. RESULTS: No patients developed any early or late complications. Six (14%) patients had a positive test: 83% revealed normal vaso-occlusive function and 33% normal arterial function. (86%) patients had a negative test: 75% revealed vaso-occlusive dysfunction and 86% abnormal arterial function. For diagnosing vaso-occlusive function, OIT had a sensitivity 83%, specificity 75%, predictive value of a positive test 36% and predictive value of a negative test 94%. For diagnosing arterial function, the injection test had a sensitivity 33%, specificity 86%, predictive value of a positive test 29% and predictive value of a negative test 89%. CONCLUSIONS: The OIT is a safe procedure. When the test was positive, there was a high sensitivity and specificity for diagnosing arterial and venous disease. When the test was positive, there was a high sensitivity for assessing normal vaso-occlusive function but a low sensitivity for assessing normal arterial function.

P3/4 – 082

CAVERNOUS NEUROTOMY CAUSES HYPOXIA AND FIBROSIS IN RAT CORPUS Cavernosum.
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The role of oxygen tension has been documented in several studies. It is known that TGF-Betas, which increases collagen synthesis in human corpus cavernosum smooth muscle cells in culture, is induced by hypoxia. We hypothesize that cavernous nerve injury can cause cavernous hypoxia due to loss of nocturnal erection and cavernous fibrosis from increased TGF-Betas. This study evaluates the effect of cavernous neurotomy on cavernous hypoxia, TGF-Betas, collagen 1 and III, pharmacologically induced intracavernous pressure response, and histological changes. Twenty male Sprague-Dawley rats (300-325 gm) constituted the study population. The animals were divided into 2 groups: 1) sham-operated rats (n=10), and 2) rats who underwent incision of bilateral cavernous nerve (n=10). Three months later, all rats underwent intracavernous pressure response studies using intracavernous papaverine injection at 300 and 600 micrograms, respectively. Hypoxic-inducible factor-1 (HIF-1 alpha) protein expression, and collagen 1 and III were studied using RT-PCR. Western blot and immunohistochemical stain. All rat penises then underwent histological examination using Hart's stain for elastic fibers and trichrome stain to determine the relative proportion of collagen to stromal smooth muscle. At 3 months, the intracavernous pressure induced by papaverine injection was similar in both groups. The expression of HIF-1 alpha, TGF-Betas, and collagen 1 and III were higher in the neurotomy group. The results of this study may support the role of hypoxia prevention for recovery of erectile function after nerve-sparing radical pelvic surgery, which may cause cavernous fibrosis due to prolonged cavernous hypoxia from neuropraxia.
P3/4 – 083

QUANTIFYING THE VALUE OF RESISTIVE INDEX IN THE DIAGNOSIS OF VENOUS INSUFFICIENCY.

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INTRODUCTION: Despite goal directed therapy for erectile dysfunction (ED), penile color Doppler (PBF) remains a popular, non-invasive method to determine the cause of ED. Redosing pharmaco-cavernosometry (RPC) is an accurate, though cumbersome, means to diagnose veno-occlusive dysfunction (VOD). In the present prospective study, we wanted to determine, through quantitative analyses, if PBF is accurate enough to be used in place of RPC for the diagnosis of VOD. MATERIALS & METHODS: In this prospective study, 122 patients who did not meet exclusionary criteria underwent intracavernosal injection test (ICI), PBF studies, & RPC testing. Flow to maintain rigidity (MF) of <5ml/min was considered evidence of an intact veno-occlusive mechanism. Quantitative analyses consisted of calculation of receiver operator characteristic curves (ROC), a mathematical function enabling quantification of the ability of variables to discriminate between outcomes. RESULTS: A total of 35 and 87 patients were good and poor ICI responders, respectively. Resistive index (RI) was normal in 94.3% of good responders, but was abnormal in only 48% of poor responders. In addition, MF was normal in 91.4% of good responders, but was abnormal in 65% of poor responders. Using MF <5ml/min as the outcome, the ROC value of RI was 0.91. CONCLUSION: Based on our analyses, RI is an excellent predictor of the absence of VOD in patients who respond to ICI with good erections. The RI, however, will not accurately detect the presence of VOD in ICI poor responders. We conclude, therefore, that determining RI, in place of RPC, is sufficient for establishing the diagnosis of lack of VOD in patients who respond to ICI.

P3/4 – 085

SMALL INTESTINAL SUBMUCOSA AS A TUNICA ALBUGINEA GRAFT. Manoj Monga, Paul Zupkas*, Danny Cosgrove*, Mahadevan Rajasekaran, UCSD Medical Center, San Diego, CA.

A variety of autologous and allogenic grafts have been used after Peyronie's plaque excision. Limitations include graft contraction, graft infection, and development of venous outflow disease. Small intestinal submucosa (SIS) has served as a successful scaffold for tissue remodeling in many organs. We evaluate the utility of SIS as a tunica albuginea substitute.

Male New Zealand white rabbits (4 kg, n=5) were anesthetized and a 4x2 mm portion of tunica albuginea excised from the dorsolateral corpora. The tunical defect was covered by a SIS graft (Surgisis, Cook Urological) and secured with a running 6-0 polypropylene suture. Three months later the rabbits were re-anesthetized. A 25G butterfly needle was used to cannulate the left corpora cavernosa for measurement of mean cavernosal pressure (MCP, cm H2O) and the right corpora cavernosa for intracavernosal drug injections. Erectile response to sodium nitroprusside (SNP, 10, 30, 100µg) and triple-drug combination (PGII, 0.5µg, papaverine [1.65µg] and phentolamine [25µg]) was evaluated. This protocol was repeated in age matched sham operated control animals. Changes in MCP was transmitted to a computerized data acquisition program.

Intracavernosal SNP produced a dose dependent increase in MCP in control as well as SIS grafted rabbits. The MCP (cm H2O) in SIS grafted animals for SNP (10µg) was 42.5 ± 2.5 compared to 43.5 ± 8.5 in control rabbits. There was no statistical difference in duration of erectile response following SNP (44 min. with 100µg), increase in MCP following triple combination (3.8 fold increase) or erect penile length between the two groups of animals.

SIS grafting of the tunica albuginea maintains the normal erectile response to vasoactive agents and is not associated with graft contracture. Ongoing studies will evaluate the histologic and molecular response to SIS grafting in the penis.

P3/4 – 086

RANDOMIZED, DOUBLE-BLIND, CROSSOVER STUDY OF THE COMPARATIVE PHARMACODYNAMICS OF TWO BIMIX AND TWO TRIMIX INTRACAVERNOSAL INJECTION FORMULATIONS IN PATIENTS WITH SEVERE ERECTILE DYSFUNCTION.


Objectives: To compare the pharmacodynamics and safety of two Bimix and two Trimix injectable in non-responders to intracavernosal injection with 20 mg alprostadil (Caverject®). Methodology: A randomized, double-blind, 4-way crossover in-office study in Mexico of 40 patients with severe ED who failed to achieve an erectile response to testing with 20 mg of Caverject(Visit1). Patients received during 4 subsequent visits an intracavernosal injection of 0.5 mL of one of the following four formulations. Patient self-assessment of the erectile response (ER), a subject symptom questionnaire, blood pressure and adverse events (AEs), monitoring were performed pre-dose, and at various time points up to 2 hours post-dosing. Efficacy analyses were based on ANOVA-type techniques for a 4x4 Latin Square Design. Primary efficacy was evaluated by erection score on a scale of 1 (no tumescence) to 4 (full rigidity). To demonstrate that at least one of the Trimix/Bimix had greater efficacy than Caverject/E. Secondary efficacy variables were as: Maximum ER; Time from injection to satisfactory response; Duration and percent of ER sufficient for vaginal penetration. Safety was assessed on monitoring of treatment-emergent AEs. Patients who did not achieve an ER (score ≥ 3) on any of the treatments were given a 1.0 mL dose of Bimix1 (Visit 6). Results: An analysis was conducted when 37 non-responders to Caverject/E had completed all study procedures. Overall, there was not statistically significant difference between the Bimix and Trimix in ER rate (Bimix1 43%, Bimix2 46%, Trimix1 46%, and Trimix2 51%).

Abstracts – Poster Session 3/4
P3/4 – 087

GENE TRANSFER OF SUPEROXIDE DISMUTASE TO THE RAT PENIS REVERSES AGE-RELATED ERECTILE DYSFUNCTION

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Nitric oxide (NO) is the principal mediator of penile erection. A decrease in erectile function can be attributed to reductions in NO activity in aged rats resulting from increased levels of superoxide. This study evaluates the role of superoxide dismutase (SOD) in improving erections in the aged rat.

The production of superoxide by membrane NADH/NAPDH oxidase activation was investigated in the corpus cavernosum of young (20 wks) and aged (60 wks) rats. Two groups of animals were transplanted with adenoviruses: 1) aged rats with AdCMVbeta1g and 2) with AdCMVbeta2. One day after transplantation, these animals underwent cavernosal nerve stimulation (CNS) and intracavernous pharmacotherapy to assess erectile function. Adenoviral transduction efficiency of Beta-galactosidase reporter gene was measured. SOD protein and mRNA levels were determined in aged rat cavernosal tissues transplanted with AdCMVbetag and AdCMVbeta2 and in young rats. Higher superoxide levels were observed in aged rats when compared to young rats. This superoxide was primarily driven by membrane bound NADH/NAPDH oxidase. One day after administration of AdCMVbeta2, SOD protein and mRNA levels in the corpora cavernosa were significantly increased (P<0.05) when compared to AdCMVbeta2 animals.

Superoxide generation was lower in aged animals transplanted with SOD when compared to those transplanted with the reporter gene. The increase in cavernosal pressure in response to CNS and intracavernosal administration of acetylcholine was enhanced in animals transplanted with SOD to levels observed in young rats. These data suggest that in vivo gene transfer of SOD can reduce superoxide levels and physiologically improve erectile function in aged rats.

P3/4 – 088

A COMPARATIVE STUDY TO EVALUATE THE VALIDITY OF NOCTURNAL PENILE TUMESCENCE MONITORING AND COLOR-CODED DUPLEX SONOGRAPHY IN THE DIAGNOSIS OF VASCULOGENIC IMPOTENCE

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The objective was to compare different measurements of color-coded duplex sonography (CCDS) and nocturnal penile tumescence monitoring (NPT) in the evaluation of suspected vasculogenic impotence to detect the accuracy of these procedures. A prospective study included 64 impotent patients with recurrent weak or absent response to intracorporeal injection (ICI) test underwent CCDS, NPT monitoring and dynamic pharmacocavernosometry-cavernosography (DPCC). The sensitivity, specificity and accuracy of NPT in the diagnosis of vasculogenic impotence were 87.2%, 82.3% and 85% respectively as compared with CCDS. However, it showed variability according to the etiological vascular factors. The best CCDS parameters could be obtained at the peno-scrotal junction within the first 10 min. after injection. In comparison with DPCC, the sensitivity of CCDS and NPT in the diagnosis of venogenic impotence were 74/4% & 74.4% respectively. The incidence of vascular risk factors was higher in both abnormal CCDS & NPT groups but with non-significant differences. Conclusion: ICI test may be reliable to expect but not conclusive test & NPT monitoring is a good valid procedure however, with lower sensitivity and specificity in predicting the type of vascular abnormalities as compared with CCDS. Also by CCDS, the diagnosis of venogenic impotence especially with borderline values of End Diastolic Velocity and Resistance index. should be insured by DPCC before management.

P3/4 – 089

SURGERY OF CORPORA CAVERNOSA BY PLICATION AND ALLODERMIC GRAFT

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OBJECTIVES: surgery of penile bending is today not more restricted only to severe deformities but may include also aesthetic defects and even minimal curvatures are more and more requested for correction.

MATERIAL AND METHODS: We perform this surgery in Hospital Day, local anesthesia and immediate discharge. For light congenital bending within 45° we perform the simple plication of convex albuginea, according to Ebbehoj-Metz technique that we modified by a "straightening-renforcing" stitch. Also in Peyronie, after pharmaco-physical cure, with normal erection but disturbed penetration due to the bending, the same procedure may be suitable. For more severe curvatures, to avoid length reduction, we prefer plaque excision and substitution with allodermic graft.

RESULTS: On the n. 36 plications (24 congenital / 12 Peyronie), and n. 12 plaque excisions with allodermic substitution, we reported, at an average follow up of one year, a complete restoring of sexual satisfaction both under aesthetical and functional point of view, assessed by clinical check and questionnaire.

CONCLUSIONS: In front of the modern sexual view of the partners, even minimal bending are today hardly accepted by young patients, increasing anxiety and stress in sexual approach. Modified plication in these cases as in Peyronie's curvatures may be a minimvasive and effective treatment, suitable for everybody. On the same bases and features, also more severe curvatures are today treatable, with allodermic graft, really safe and effective.

P3/4 – 090

SPECIFIC AGGLOTTINATION OF HUMAN SPERM BY CHICKEN ANTIBODIES AGAINST HUMAN SPERM FROM EGG YOLKS OF IMMUNIZED HENS

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Chicken IgY's were postulated by us to neutralize sperms ejaculated into virginia without activating complement system and would be a candidate of ideal contraception [NQ Lu, YF Wang, YF Huang et al. Asian J Androl. 1999, 1(3):87-93]. To test this hypothesis, laying hens were regularly administered by intramuscular injection with washed human sperm at an one week interval. Immunized eggs were collected after 4 booster injections for isolation of yolk immunoglobulins. The yolk was separated from the white and carefully washed with Tris-buffered saline (TBS) (1 μM Tris, 14 μM NaCl, pH 7.4) to remove as much of the albumin as possible. For one yolk (15 ml), 40 ml of TBS were added and homogenized for ten min with a mixer. Then 40 ml of chloroform were added with continuous stirring. Immunoglobulins were precipitated by ammonium sulphate solution and passed through a Sephadex G-25 column to remove sulphate. The presence of anti-human sperm antibodies in the extracts was confirmed by sperm agglutination test. The control test with non-immunized chicken IgY and chicken sera showed negative results in sperm agglutination test. In conclusion, the phylogenetic distance between birds and mammals, the ability of chickens to produce high levels of specific antibodies following immunization with human sperm, and the transfer of large amounts of these antibodies from the serum of the laying hen to the yolk of the unfer-tilized egg have encouraged the development of immunoglobulins-based passive immunococontraceptives in the near future.
**P3/4 – 091**

**THE ROLE OF IMMUNOSUPPRESSIVE TREATMENT IN ADOLESCENTS AND YOUNG MEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN THEIR SEMEN ANALYSIS**

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Steroids and immunosuppressive therapy are necessary for controlling the Juvenile Systemic lupus erythematosus activity (SLE) irrespective the possible side effects that may occur as oligospermia or even azoospermia. We sought to evaluate adolescents and young men with SLE who underwent immunosuppressive therapy. Four young men with SLE were submitted to a clinical and laboratorial (testicular ultrasound and semen analysis) investigation following immunosuppressive treatment. All patients were asked to provide 3 semen samples following masturbation after a minimum of 3 days sexual abstinence. All 4 patients had severe disease with renal involvement and were treated with prednisone, methotrexate, cyclophosphamide and prednisone. The median follow up after treatment was 6 years and 7 months. The median patients age for beginning to ejaculate was 13.7 years. All patients had a normal erection, libido, physical examination including testicular volume and normal testicular ultrasound. One patient was azoospermic, another oligospermatic and two were teratospermic. The two patients with a better semen analysis have a bigger follow up (8 years and 9 years). Seminal morphology abnormalities were observed in all patients Despite the small number of patients, it seems that the immunosuppressive treatment for patients with SLE may damage the testicle function. Further semen analysis will demonstrate if these alterations are transitory or definitive.

**P3/4 – 092**

**THE BLOOD-TESTIS BARRIER IS NOT A BARRIER TO SPERM ANTIBODIES IN THE MINK (Mustela vison).**

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Up to 20-30% of male mink experience primary or secondary infertility. Puberty is indefinitely delayed in primary infertility. In the secondary infertility, males spontaneously develop pathologic features consistent with an inflammation of the testes called autoimmune orchitis (AOI) that leads to infertility. The present study assesses the relation between the changes in the permeability status of the blood-tissue barriers of the male reproductive system to variously infused blockers and the changes in 1) the content of the germ cells and in 2) the serum levels of anti-sperm antibodies (Ab) measured by ELISA and immunofluorescence microscopy during post natal development and during the annual reproductive cycle of the adult mink. The results show that periods of transient permeability of the barrier were not accompanied with significant increases in serum anti-sperm Ab in normal mink. Infertile adult mink suffering from AOI showed a transient increase of their serum anti-sperm Ab. In addition, serum testosterone levels were transiently lower in infertile than in fertile mink. There was no significant difference in intratubular Fas ligand levels between fertile and infertile mink. Fas intratubular levels were significantly higher in fertile than in infertile mink. Apoptosis measured by cell death detection ELISA was maximal coincidentally with the disappearance of the spermatocytes and round spermatocytes. The result show that the blood-tissue barrier is not a barrier to antisperm Ab. Support NSERC to RMP and MLV; Population Council to RMP. SR Yoon is a Hansol fellow.

**P3/4 – 093**

**THE CHARACTERIZATION OF HUMAN SPERMATOZOA ANTIGENS AND IMMUNOLOGICAL INFERTILITY.**

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Antisperm antibodies (ASA) are the main cause of immunological infertility, which impair sperm functions by binding on the sperm membrane. In this study we isolated highly enriched sperm membrane proteins by two-dimensional gel electrophoresis. Isoelectric focusing, as a first dimension, was performed on precast DryStrip IPG 4 - 7. The second dimension was carried out on 12 percent SDS gels. A total of 18 antigens were identified by the subsequent two-dimensional western blot using ASA from seminal plasma of infertile patients, determined with the MAR test (mixed antiglobulin reaction). Six of the recognized proteins were isolated and analyzed by means of mass spectrometry and peptide matching. They were identified as heat shock proteins HSP70 and HSP70-2, the disulfide isomerase ER60, the inactive form of caspase-3 and two subunits of the proteasome (component 2 and zeta chain). The biochemical identification of these proteins will be helpful to understand the mechanism by which ASA impair sperm function and the fertilization process. Thus they are maybe candidates for contraception and for the development of reliable methods for ASA detection.

**P3/4 – 094**

**IDENTIFICATION OF ANTIBODIES AGAINST SPERMATOZOA IN SERUM OF MALE WILD FOXES.**

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The sera from wild male foxes (Vulpes vulpes) were collected during the time of tests production and were analysed in order to find antisperm antibodies. Serum samples were obtained from 76 wild foxes shot in the north-east of France (for evaluating rabies vaccination program). Western blot with these sera diluted at 1/2000 were performed on SDS-PAGE on protein extracts from fox spermatozoa. Then positive sera were checked by Western blotting on different tissues (liver, brain, spleen). Antibody binding to spermatozoa surface was visualized by indirect immunofluorescence on epididymal spermatozoa. 36 out of 76 sera revealed protein bands by western blotting in reducing conditions. We observed a wide range of molecular weights ranging from 19 to 120 kDa. Only 16 out of the 36 positive sera showed no cross reaction with liver, brain and spleen proteins and reacted only with spermatozoa specific proteins. Among these 16 sera, 10 have been able to recognize surface components of spermatozoa according to the immunofluorescence technique. Our results support evidence that some wild male foxes develop spontaneously a specific autoimmune response against proteins from spermatozoa during the testis activity. Future investigations will focus on the characterization of these sperm proteins.
P3/4 – 095
EVIDENCE FOR IMMUNOSUPPRESSIVE EFFECTS OF SEMENOGELIN, MAJOR PROTEIN OF SEMEN COAGULUM
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Semenogelin is secreted from human seminal vesicle and constitutes a major gel-forming protein in semen. Semenogelin plays an important role for inhibiting sperm motility. It has been reported that human seminal plasma has immunosuppressive effects on human peripheral blood lymphocytes (PBL). We have studied whether human semenogelin exerts immunosuppressive effects on human lymphocyte functions in vitro. We found that semenogelin reduced mitogen phytohaemagglutinin (PHA) and anti-CD3 monoclonal antibody induced proliferation of PBL. Rabbit polyclonal anti-semenogelin antibody, which neutralizes sperm motility inhibitory effect of semenogelin, reversed the immunosuppressive effect, indicating that semenogelin is responsible for the effect. IL-2 secretion and IL-2 messenger RNA expression by PBL were reduced by the semenogelin treatment. Polyclonal immunoglobulin production induced by pokeweed mitogen (PWM) stimulation was also inhibited by the semenogelin treatment. Collectively, semenogelin has suppressive effects on the T cell-mediated immune responses and immunoglobulin production in vitro. Semenogelin may be associated with immunological acceptance of sperm in the female reproductive tract at fertilization by reducing anti-sperm immune responses.

P3/4 – 096

Cytokines are immune messengers involved in the host defense against pathogens. In this study we determined tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in the search of seminal markers of male genital infection in asymptomatic infertile men. We also investigated their relationship with the number of peroxidase white blood cells (PMN). The microbiological study was performed following Stamey and Meares. Cytokines were determined by ELISA and expressed as median and range. We evaluated 49 asymptomatic infertile men without varicocele (aged 20 to 45 years old) and 16 fertile donors (C). We detected 20 patients with infection (1 -):11 with gram + -positive organisms, 2 with gram -negative organisms and 7 with Ureaplasma urealyticum. In group C: IL-6 was 34.0 (15.0-65.50 pg/ml), TNF-α was 13.0 (5.0-20.0 pg/ml) and PMN/ml 167,500 ± 156,098. In view of two different levels of cytokines and PMN found in I, we defined two subgroups 1' and 2'. In 1' (n:10) the cytokines and PMN were similar to non infected men and C group. In 2' (n:10) IL-6 was 150.0 (70.0-804.0 pg/ml); TNF-α 49.0 (20.0-200.0 pg/ml) and PMN/ml 1,225,800 ± 63,692 (p < 0.01 in all cases vs C). A positive correlation was found between the concentration of cytokines and PMN (r=0.66; p=0.04). Eight patients from I2' group underwent antibiotic treatment. Cytokines and the number of PMN was significantly reduced after bacteriological cure. Bacterial infection is not always associated to an inflammatory seminal pattern (high levels of IL-6 and TNF-α with leukocytospermia) in asymptomatic infected infertile male.

P3/4 – 097

The injection of plasmid DNA induces a systemic humoral immune response to the encoded antigen. It has proved to be advantageous in production of antibodies, compared to traditional protein immunization. This study was aimed at evaluating the production of antiacrosin antibodies in mice inoculated with the cDNA encoding the human proenzyme, and compare it with that obtained after immunization with a truncated product of proacrosin (Rec-30, residues 1-300) produced in bacteria. The cDNA encoding h-proacrosin (Adham et al., 1989), was subconed in the eukaryotic expression vector pSF2 containing the α-1 antitrypsin signal peptide, and the CMV promoter (pSF2-Acro). Recombinant proacrosin (Rec-40) and Rec-30 were obtained using preparative electrophoresis of bacterial lysates (Furlong et al., 2000). Female Balb/c (Two) mice were inoculated with pSF2-Acro (10, 20, 40 ug; im) or Rec-30 (20 ug; sc/p) 4 times every three weeks. Plasmid pSF2 and buffer were used as controls. Production of antibodies was analyzed using an ELISA with Rec-40 as antigen, starting at week 6 of the immunization protocol. Antibody levels were significantly higher (p<0.0001) in animals inoculated with DNA purified using ionic exchange columns, comparing with phenolchloroform purification. A similar response was obtained throughout the time (5-15 w) when injecting either protein or DNA. The highest signal was obtained at week 9 of the protocol. Ten ug of DNA were sufficient to give a specific response. The titer decayed to a 50% of the maximum value 6 months after protocol initiation. Gene immunization with the proacrosin cDNA results in a specific high response towards the protein. The model will be used to study proacrosin role(s) in fertilization.

P3/4 – 098
INTERLEUKIN-1β, INTERLEUKIN-6 AND ANTI-SPERM ANTIBODIES IN SEMINAL PLASMA OF INFERTILE MEN WITH AND WITHOUT GENITIL INFECTION
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Objectives: To determine the effect of genital tract infection on the release of pro-inflammatory cytokines [Interleukin-1β (IL-1β), Interleukin-6 (IL-6)] and on the immune system formation of anti-spem antibodies in seminal plasma of infertile men (ASA).

Patients & Controls: 55 infertile patients,20 normal controls.

Setting: Andrology Clinic, Assiut University Hospital, Egypt.

Methods: Semen analysis and semen cultures. Seminal levels of IL-1β and IL-6 and of ASA were measured by ELISA techniques.

Results: 25 (45.5%) infertile patients had positive cultures (≥ 10^3 colonies /ml). Infertile patients with and without infection had significantly lower sperm concentration, % of motile sperms and % of viable sperms and higher % of abnormal forms compared to controls (P < 0.001). Infertile patients with infection showed significant increased seminal levels of IL-1β, IL-6 and ASA compared to infertile patients without infection (P < 0.05, P < 0.001 and P < 0.05 respectively) and controls (P < 0.001), whereas, the infertile group without infection showed only significant increase of ASA compared to controls (P < 0.001). There were no significant correlation between the number of infecting colonies / ml and sperm or measured parameters.

Conclusion: Genital infection results in elevated levels of seminal pro-inflammatory cytokines (IL-1βand IL-6) and of seminal ASA, both of which may be critically involved in male infertility.
P3/4 - 099

**ORCHIDIC TOLERANCE WORKS IN THE MOUSE AS WELL**
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**INTRODUCTION:** Systemic tolerance induced by intratesticular antigen injection prior to immunization with the same antigen is a phenomenon that has been originally introduced and studied using experimental autoimmune uveoretinitis (EAU) (Peng et al., 1992; Ren et al., 1994, 1995, 1996) in the rat. Later the phenomenon has been named orchidic tolerance. In this study, it was investigated if the orchidic tolerance can be induced in the mouse as well and the phenomenon is testis specific.

**MATERIALS AND METHODS:** Five mice (T) were given an intratesticular injection of MSCH (Mouse Spinal Cord Homogenate) 96 and 48 hours before the induction of EAE (Experimental Autoimmune Encephalitis), five mice (T) were given an intratesticular injection of PBS and five mice (M) were given MSCH injections into the hamstring muscles. EAE was induced to all mice and then they were followed for 30 days and graded for clinical symptoms (0-5) on daily basis.

**RESULTS AND DISCUSSION:** Mice (T) treated with an intratesticular injection of MSCH got sick latest, had the mildest symptoms and the shortest duration of symptoms. This proves that the phenomenon is testis specific but not rat specific.

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P3/4 - 100

**SPERM MORPHOLOGY AND NUCLEAR DNA INTEGRITY AFTER DENSITY GRADIENT CENTRIFUGATION (DGC) THROUGH PURESPERM™: RELATIONSHIP TO IVF OUTCOME**
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**Introduction**
The relationship between ART outcome and sperm nuclear DNA integrity has been the focus of much recent attention. Here, we examine further the interrelationships between conventional semen parameters and sperm DNA/chromatin integrity, before and after DGC, to determine whether sperm DNA assessment is of prognostic value.

**Materials & Methods**
Samples obtained from our ART program were assessed using WHO guidelines, with DGC, carried out using 0.5m volumes of PureSperm™ (Nidacol, Gothenburg, Sweden). The 90% sperm pellet was fixed (3.5% formaldehyde). 3 smears were prepared and left to air dry and 3 more prepared from the raw semen. DNA integrity was assessed using In situ nick translation (NT) and Chromomycin A3 (CMA) assays (Manicardi et al., 1995).

**Results**
Sperm morphology and DNA integrity improved after DGC (P=0.0001). NT and CMA3 were negatively correlated with sperm concentration and positively correlated with each other (P=0.0001). Normal forms in the raw sample were negatively correlated with NT (P=0.01) and CMA3 (P=0.05) were both significantly higher in oligospermics, with 5% normal forms significantly lower. Pregnant patients had significantly lower NT values (post DGC) when compared to the non-pregnancy group.

**Conclusion**
DGC enriches the sample, improving morphology and nuclear integrity. DNA integrity appears to be related to the establishment and continuation of pregnancy and further studies will demonstrate whether semen analysis is of more prognostic value, after DGC, rather than before.

**References**

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P3/4 - 101

**SERUM INHIBIN β CANNOT PREDICT TESTICULAR SPERM RETRIEVAL IN PATIENTS WITH NON-OBSTRUCTIVE AZOOSPERMIA.**
V Vernaeve*, H Tournaye, J Schiettecatte*, G Verheyen*, A Van Steirteghem and P Devroye*, Centre For Reproductive Medicine, University Hospital, Dutch-speaking Brussels Free University, Belgium.

**Introduction:** The present study aimed at evaluating the predictive value of inhibin β for retrieving testicular spermatozoa in non-obstructive azoospermic men. Materials and methods: We reviewed the serum inhibin β value of 185 non-obstructive azoospermic patients before sperm retrieval. The discrimination between successful and unsuccessful sperm retrieval was analyzed using the receiver operating characteristic curve analysis. Results: 128 patients out of 185 (69.2%) showed complete or incomplete germ-cell aplasia (Sertoli cell-only), 49 patients (26.5%) showed complete or incomplete maturation arrest and 8 patients (4.3%) showed tubular sclerosis and atrophy. In 92 out of these 185 patients (49.7%) testicular spermatozoa were successfully recovered. In 93 patients (50.3%) no spermatozoa were found. The mean inhibin β value in patients with sperm was 37.28 pg/ml and 44.99 pg/ml where no sperm was found. The best discriminating inhibin β value was 13.7 pg/ml (sensitivity 44.6 %, specificity 63.4 %) with an area under the ROC curve of 0.51. Conclusion: Our analysis shows that inhibin β fails to predict the presence of spermatozoa in men with non-obstructive azoospermia undergoing TESE.

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P3/4 - 102

**SPERM RETRIEVAL AND FERTILIZATION IN REPEATED PERCUATANEOS EPIDIDYMAL SPERM ASPIRATION.**
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There has been a fear that the percutaneous epididymal sperm aspiration (PESA) procedure, being blind, could cause a damage to the epididymal duct system and make it impossible to retrieve spermatozoa if a repeated procedure is required. We report here on repeated PESA procedures from the same unilateral epididymis. The records of 20 patients (23 attempts) who underwent a repeat PESA procedure from January 1996 to September 2000 for assisted reproductive techniques purposes were reviewed. In all patients the repeat procedure was performed at the same side of the previous PESA.

Data were collected on patients age, presence of motile sperm during the PESA procedure, epididymal side, pregnancies and abortion rates. The mean age of the patients was 32.4 ± 5.6. One patient was excluded from our analysis due to the lack of information regarding the side of the procedure in the chart. Repeat PESA was performed in the right epididymis in 12 attempts and in the left in 10. Of the remaining 19 patients, 14 (73.68%) did not have sperm present on the epididymal fluid and 5 (26.3%) did. In these 5 patients, 8 repeat PESA procedures were performed (1 patient was submitted 3 times to a repeat PESA and another 2) and motile sperm was always found (8/12: 36.4%). Three patients got pregnant with the motile sperm retrieved from the repeat PESA (3/3; 37.5%). There were no abortions detected. This study shows that in men with obstructive azoospermia, PESA can be repeated on the same unilateral epididymis with good opportunity of retrieving sufficient motile spermatozoa for ICSI.
Abstracts – Poster Session 3/4

P3/4 – 103
CUMULATIVE PROBABILITY OF PREGNANCY AND IMPLANTATION RATES ARE SIMILAR FOR THE FIRST FOUR EMBRYO TRANSFERS (ETS) (FRESH OR FROZEN) FOLLOWING IN VITRO FERTILIZATION (IVF) WITH INTRACYTOPLASMIC SPERM INJECTION (ICSI).


Recent studies suggest that ICSI does not decrease the chance of success for frozen ET. Patients who fail to conceive following their first oocyte retrieval with fertilization by ICSI need to make a decision as to whether to spend more money and go through the risk of ovarian hyperstimulation and oocyte retrieval or do a frozen ET. To make intelligent decisions, e.g., switching to therapeutic donor insemination, the couple needs to know what the success would be if there had been failure to conceive after 1, 2, or 3 previous transfers. For this reason we studied the cumulative probability of pregnancy for up to 4 consecutive IVF-ET cycles with ICSI performed for male factor. Transfers could be either fresh or frozen. The clinical pregnancy rate (PR) for the first 4 cycles were similar with a slight decrease in cycle 2 (44.0% [61/366]; 31.9% [44/138]): 45.2% [14/31]; 44.4% [4/9]). Delivery rates were also similar. There was a lower PR on the second retrieval vs the first retrieval (47.9% vs 29.1%) but this may be related to most of the second retrievals occurring in the second transfer cycle (67.2%, 31/55); this may bias a selection of women not making as many oocytes thus requiring another retrieval without a frozen ET. The majority of transfers in cycle one were fresh whereas cycles 2-4 used primarily frozen-thawed embryos.

P3/4 – 104
PERCUTANEOUS ASPIRATION BIOPSY USING AN INTRAVENOUS CATHETER: A NEW MODIFICATION FOR SPERM RETRIEVAL IN AZOOSPERMIC PATIENTS UNDERGOING ICSI.


Introduction: Needle aspiration can be used to obtain testicular spermatozoa from azoospermic patients undergoing intracytoplasmic sperm injection (ICSI). In this study we describe a new modification using wide bore intravenous catheter comparing it with classic fine needle aspiration FNA. Materials and methods: Seventy-three patients with obstructive azoospermia undergoing ICSI were included. Under local anesthesia, 31 patients underwent FNA using butterfly needle (size 21 – 23 G). A twenty mL syringe was used to create a negative pressure while withdrawing the needle. For 42 patients, an intravenous catheter (size 16 or 18 G) was used instead of the butterfly needle. When testicular tissue was seen coming out, the catheter was clamped and removed gently from the testis. Results: Successful sperm recovery was achieved in 41 patients (97.6%) undergoing aspiration by catheter compared to 16 patients (51.6%) undergoing FNA (P < 0.001). The amount of aspirated tissue allowed for cryopreservation of excess spermatozoa in all patients undergoing needle aspiration by catheter compared to only 6 (37.5%) undergoing FNA. Conclusion: The use of an intravenous catheter for testicular aspiration biopsy improved significantly the sperm recovery rate and allowed for cryopreservation of excess tissue in patients with obstructive azoospermia.

P3/4 – 105
THE COMPARISON OF THE EFFICACY OF ICSI USING EITHER SPERMATOZOA ASPIRATED MICROSCOPICALLY FROM THE EPIDIDYMIS (MESA-ICSI) OR OBTAINED FROM THE EJACULATE.


Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection (MESA-ICSI) is the viable alternative of the treatment of couple sterility caused by obstructive azoospermia. Because there are structural differences between epididymal and ejaculated spermatozoa and it is not univocally stated, if this fact influences positively or negatively the results of ICSI, it was decided to compare the efficacies of ICSI using both the kinds of spermatozoa at the same setting. The MESA group consisted of 27 couples and the control ICSI group of 154 couples - both matched by age, duration of sterility, health status, stimulation protocols and luteal supplementation. The trial was performed at the same period in one center, keeping the same laboratory and personal conditions in both the groups. In the MESA group the fertilization rate was 53.9%, the cleavage rate 87.6% and pregnancy rates 37.0% per microinjection and 43.5% per embryo transfer. In the control group the corresponding values were 50.1%, 90.0%, 28.0% and 31.7%. The differences between the groups were insignificant. It may be concluded, that the structural differences between epidiymal and ejaculated spermatozoa do not significantly influence the efficacy of ICSI, however further investigations are still needed.

P3/4 – 106
REAL TIME SPERM MICRO-SEPARATION TECHNOLOGIES FOR HUMANS AND FARM ANIMALS.


Various methods have been applied to isolate high-quality sperm for humans’ or farm animals’ reproductive purpose, including Swim Up, 2-Layer Density Column, Washing, Albinum Column, Glass Wool Filtration and Percoll Gradient. However, none of them could separate from original ejaculum a high proportion of grade 3 motile sperm, which must be morphologically normal, maintaining normal-chromatin with functional integrity of plasmalemma, yet free of less-motile and non-motile sperm, free of contaminating bacteria, debris, seminal plasma, somatic cells and other impurities. Real Time Micro-separation System (RT) is based on the principles: (1) non-pathological spermatozoon do not transfer organisms; (2) the motility pattern and swim-up capacity of pathological or infected sperm are limited; (3) the movement of sperm differs from passive motion of non-ciliated microorganisms and that of random active motion of ciliated microorganisms in terms of velocity and direction; and (4) that tissue culture medium is low viscous does not favor the adhesion of microorganisms to highly motile sperm. Our studies revealed that RT could isolate organisms-free without supplementation of antibiotics or even with inoculation of experimental bacteria, ds-DNA, morphologically normal, and grade 3 motile sperm with integrity plasmalemma. Recent study further showed that RT retrieved the highest percent of fully hypo-osmotic swelling sperm. Besides, RT encompasses a micro-separation tube, which is a unique instrument providing migrating sperm and practitioner’s procedure directly monitored via microscopy in the process of preparation. In conclusion, RT appears the gold standard of sperm selection for humans as well as farm animals, e.g., cattle, water buffalo, sheep, goat and horse.
P3/4 – 107

COMPARISON OF FOUR SPERM PROCESSING METHODS: EFFECT ON RECOVERY, CLEAN-UP, MOTILITY, AND MOTION PARAMETERS.

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Objective: To compare the Enhance (Conception Technologies, San Diego, CA), IsoCare (InVitroCare, San Diego), SpermPrep (ZDL, Lexington, KY), and swim-up ZSC-II (ZDL) sperm preparation methods with respect to recovery (percent motile sperm), recovery efficiency, clean-up (presence of debris or round cells), and motion parameters. Design: Comparison of four commercial sperm processing methods. Setting: Private medical center-based andrology laboratory and infertility program. Patients: Twenty men who presented for routine semen analysis. Interventions: Semen specimens were divided and prepared using four different processing methods evaluated by CASA. Main Outcome Measures: Recovery efficiency, percent motility, debris/round cells, and motion parameters (path velocity, progression and percent hyperactivation) before and after separation of the four different processing methods. Results: Motile recovery efficiency was greatest in Enhance (47%; P<0.01) and lowest in the ZSC-II (15%). Percent motility was greatest in ZSC-II (83%; P<0.01) and lowest in SpermPrep (46%). ZSC-II was significantly greater (P<0.05) in percent motility and percent hyperactivation than all other methods except for Enhance. There was no significant difference between the methods with respect to progressive motility. Conclusion: The Enhance washing method consistently had either equal to or superior end products across most categories. The SpermPrep sperm washing method consistently had inferior end products. Considering the relative short processing time and cost, Enhance is ideal for routine sperm washing.

P3/4 – 108

SEMINAL TRACT WASHOUT TO TREAT INFERTILITY IN ANEJACULATING PATIENTS.

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INTRODUCTION. Seminal tract washout (STW) allows to recover from the bladder (previously emptied and washed with Ham’s F10 medium) the spermatozoa of the seminal tract downstream from the epididymes. The spermatozoa may be used for in-vivo or in-vitro ART procedures or cryopreserved. MATERIALS AND METHODS. Thirty-six STW’s have been applied to 34 patients suffering from anejaculation due to spinal cord injury (18), juvenile diabetes (4), retroperitoneal lymphnode dissection consequent to monolateral testicular cancer (5), previous surgery performed for dolichomegacolon (1), imperator anus (1) and bladder extrophy (1), chronicuse of antidepressants (2), psychogenic anejaculation refractory to any treatment (2). In 14 couples spermatozoa were used for 22 stimulated ART cycles (11 fresh, 11 cryopreserved). In 20 men the spermatozoa are presently cryopreserved. RESULTS. STW allowed to retrieve forward motile spermatozoa in 33/36 men (median count 146 x 10⁶, range 0.1-7000, median forward motile 12.7%, range 0-30%). Seven pregnancies in 7 couples resulted in the live birth of 11 healthy children (2 out of 11 ICSI-cryo, 1 out of 5 ICSI-fresh; 1 out of 5 IVF; 2 out of 3 IFF; 1 out of 1 IUI) (success rate: 50% per couple; 32% per transferred cycle). CONCLUSIONS. STW is rather inexpensive, is performed in local anesthesia and does not require any microsurgical experience. In comparison to testicular sperm extraction and testicular fine needle aspiration, STW recruits a larger number of motile spermatozoa, sometimes fit even for IVF, and with better chances of cryopreservation.

P3/4 – 109

EFFECT OF SEMEN PROCESSING ON CHROMATIN, MORPHOLOGY VITALITY, MOTILITY AND ITS RELEVANCE TO CRYOPRESERVATION.

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Purpose: The aim of this study was to find out if separation of spermatozoa (swim-up) before cryopreservation improve post-thaw vitality, motility, chromatin integrity and morphology in comparison to unprocessed (native) cryopreserved sample. Material and Methods: 30 semen samples were subdivided into two aliquots. The first aliquot (G.1) frozen and thawed after mixing with Human sperm cryopreservation medium (HSPM, 1:1). Whereas the second aliquot (G. 2) was first processed by swim-up technique and the supernatant, which include motile spermatozoa was mixed (1:1) with HSPM and then frozen. Freezing procedure was carried out using liquid nitrogen vapour. Chromatin integrity was evaluated after staining with acridine orange. Morphology was scored according to strict criteria. Vitality was analysed after staining with eosin test. Results: In the first group, the mean percentage of condensed chromatin, morphologically normal, and motile spermatozoa was (83.10±6.7%; 19.2±1.4% and 50.6±23.0% respectively). These values decreased after freeze-thawing to (30.0±22.6%, 9.6±4.8%, and 15.5±14.2%) respectively. However, after semen processing of the second aliquot, the mean percentage of condensed chromatin, morphologically normal, and motile spermatozoa was increased to (87.2±13.3%, 21.2±10.0%, and 71.1±26.2%) these parameters, in turn, decreased to (30.2±14±26.5%, 14.4±8.2% and 24.3±19.26% respectively). Native and processed cryopreserved spermatozoa showed statistically significant decrease in chromatin condensation and motility after freeze-thawing. The main percentage of chromatin and motility croydamage of raw semen sample were significantly higher in comparison to those values found of processed semen.

P3/4 – 110

COMPARISON BETWEEN SPERMATOZOA QUALITY (MORPHOLOGY AND CHROMATIN INTEGRITY) AFTER SEMEN PROCESSING WITH PURE SPERM OR GLASS WOOL FILTRATION AND THEIR EFFECT ON IVF OUTCOME.

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Objective: The aim of this study was to compare the recovery rate of morphologically normal and chromatin condensed spermatozoa after semen processing either with pure sperm or glass wool filtration and to find out the influence of two processing techniques on fertilization and pregnancy rate of in an IVF program. Material and Methods: Semen samples (n=101) obtained from IVF patients husband. The semen samples were evaluated according to WHO criteria except for morphology which was assessed according to strict criteria. The semen samples were processed either with pure sperm (G.I, n=63) or with Glass wool filtration (G.II, n= 38). From each semen sample many smears were made before and after semen processing. The chromatin condensation was evaluated after staining the smears with aniline blue staining. The recovery rate of morphologically normal and chromatin condensed spermatozoa as well as the fertilization and pregnancy rate were compared between the two groups. Results: The main percentage of morphologically normal and condensed spermatozoa in the G.I was (4.5±4.6%, 31.8±20.9% respectively) and the corresponding values in the second group G.II were (3.2±4.2% and 33.8±21.2%). After semen processing there was a significant increase in the main percentage of morphologically normal and condensed spermatozoa not only in the G.I (13.7±10.8% and 46.4±26.0%) but also in G.II (8.3±7.1% and 45.3±24.2%). However, the recovery rate of morphologically normal spermatozoa was significantly higher (p=0.002) after semen preparation with pure sperm in comparison to glass wool filtration technique.
P3/4 – 111

ICSI IN AZOOSPERMIA WITH SURGICAL SPERM RETRIEVAL – PREDICTORS OF OUTCOME.

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Is it possible to predict ICSI with nonejaculated sperm and what is the optimal work-up in azoospermia? Material & Methods. 52 patients with obstructive (OA) and 123 with nonobstructive (NOA) azoospermia underwent ICSI. In 55 OA cycles Percutaneous Epididymal Sperm Aspiration (PESA) was done and in 142 NOA cycles Testicular Sperm Extraction (TESE). In all OA and 40.6% NOA, sperm was found and ICSI done. Remaining sperm was frozen allowing ICSI with thawed sperm, epididymal, in 81 cycles, and testicular in 67 cycles. Uni and multifactorial analyses were done using simple, logistic and stepwise statistics. Results: In NOA, male age, FSH or tests histology failed to predict TESE. In OA and NOA, pregnancy, correlated only to wife age (p=0.005), retrieved (p=0.016) and injected oocytes (p=0.0003). Comparing OA and NOA, embryological outcome and pregnancy was similar but abortions increased in NOA (p=0.012) and aged wives. Using fresh or thawed sperm, ICSI outcome was similar in NOA, but not in OA with less fertilizations (p=0.03) with thawed sperm. Conclusions: In OA, as sperm retrieval is mostly possible and ICSI fertilizations decrease with thawed sperm, ICSI should be planned with fresh PESA. In NOA, as sperm retrieval predictors are not efficient, sperm retrieval rate is lower but ICSI outcome is similar with fresh or thawed sperm, elective TESE might be offered prior to ovarian stimulation, mainly when patients exclude donor sperm backup. Once non-ejaculated sperm is available, only female factors bear significant impact upon ICSI success and subsequent pregnancies. Female age >38 years and diminished ovarian reserve with < 4 mature available oocytes do compromise ICSI outcome.

P3/4 – 112

PREDICTIVE VALUE OF SERUM INHIBIN-B IN THE TESTICULAR ESPERMATIC RECUPERATION IN TESTICULAR AZOOSPERMIA

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Objective: To study the predictive value of serum inhibin B, as a marker of spermatogenesis, in the recovery of spermatic function in males with testicular azoospermia, towards a potential indication to treatment by FIV-ICSI or IAD

Patients and methods: 34 patients have been studied. Group I: patients affected by testicular azoospermia (n=17). Group II: patients with posttesticular azoospermia (n=17). The levels of FSH, B inhibin, and testicular volume were determined.

Results: FSH levels were significantly higher and inhibin B was significantly lowered in the patients of group I compared to group II. Inhibin B was detected as significantly higher in the patients of group I from which spermatozoa were recovered (n=10) as compared to the patients from the same group were the recovery was negative (n=7). however no significant differences were detected when comparing the levels of FSH and testicular volume. Among all parameters studied in group I, only inhibin B (>40pg/ml) discriminated towards the prediction of recovery or not of spermatozoa from testicular biopsy.

Conclusion: Inhibin B is a simple and non agressive method, which has a higher predictive value of the recovery of spermatozoa from testicular biopsy as compared to FSH and testicular volume.

P3/4 – 113

GENETIC STUDY OF 500 STERILE COUPLES IN ICSI PROGRAME DUE TO SEVERE MALE FACTOR

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Objective: To determine the incidence of genetic anomalies in both members of sterile couples due to severe male factor and to evaluate the convenience to perform systematically this type of studies before to enter in ICSI programe.

Patients and Methods: 500 sterile couples due to severe male factor will be included in the study. 337 of them (67.4%) being severe oligozoospermia (sperm count <10^6)ejaculate) and 163 (32.6%) being azoospermias of which 42 (8.4%) are excretory and 121 (24.2%) are secretory. A karyotype was performed in both members of each couple and, in addition, a complete molecular study of the Y chromosome was performed on all male partners.

Results: Among oligozoospermic patients 7 (2.07%) chromosomal anomalies were detected. These included one (0.29%) 47XXY (Klinefelter’s syndrome), two (0.59%) 47XXX, four (1.18%) equilibrated translocations. Among secretory azoospermias 10 (8.26%) karyotype anomalies, 7 (5.78%) Klinefelter and 3 (2.47%) equilibrated translocations were detected. In the group of patients affected by excretory azoospermia no chromosomal anomalies were detected but 19 (45.2%) were carriers of a cystic fibrosis mutation. In reference to the molecular study 16 (3.2%) microdeletions were detected, 4 (1.18%) in the severely oligozoospermic group and 12 (9.91%) in the secretory azoospermic group. Karyotype study in women revealed 4 (0.8%) equilibrated translocations in patients previously classified as normal.

Conclusion: This study strengthens the convenience to perform genetic analysis to all sterile couples and the necessity to realize these studies before performing complex assisted reproduction techniques, especially before ICSI.

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P3/4 – 114

HOW PREDICTIVE IS THE MOCK CYCLE DURING IVF, SHOULD IT BE PERFORMED? 1PM Zavos, JR Correa, AM Sultan, H Kaskar, 2PN Zarmakoupis-Zavos. 1The Kentucky Center for Reproductive Medicine and IVF, and, 2Andrology Institute of America, Lexington, Kentucky; Lexington, KY, USA

Objective: The demand for prediction of potential fertility in ART procedures over the years, has necessitated the development of better and more accurate predictors for sperm quantitative and qualitative characteristics. Therefore, the objective of this study was to establish the predictivness and accuracy of a mock semen preparation for IVF via direct comparison with the actual semen preparation on the day of IVF.

Materials and Methods: Male patients (n=26) whose wives were undergoing IVF treatment at our facilities were included in the study. Two semen samples were collected (within 30 days) via a non-spermicidal condom at intercourse and prepared using a conventional swim-up method on both occasions. Samples were evaluated manually according to WHO standards for volume, concentration, motility, grade of motility and critical morphology. The semen characteristics between the two samples were compared, as well as the outcome of the swim-up procedure and 24hr survival.

Results: The results obtained are shown below:

<table>
<thead>
<tr>
<th>Semen Treatments</th>
<th>Volume (mL)</th>
<th>Conc. (x10^6)</th>
<th>Motility (%)</th>
<th>Grade (4-0)</th>
<th>Morph. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock (IVF)</td>
<td>2.9 ± 1.3</td>
<td>120 ± 80</td>
<td>67 ± 15.2</td>
<td>2.9 ± 1.3</td>
<td>10.7 ± 4.8</td>
</tr>
<tr>
<td>Post IVF</td>
<td>1.0 ± 0.1</td>
<td>13 ± 8</td>
<td>90 ± 1.1</td>
<td>1.4 ± 0.6</td>
<td>8.4 ± 5.2</td>
</tr>
</tbody>
</table>

Sperm characteristics assessed (n=26)

No significant differences (P<0.05) were noted between the two raw semen specimens collected (mock v/s day of IVF).Regression analysis showed significant correlations between the semen parameters tested in both the mock and day of IVF samples. A significant difference was noted in the 24hr survival of sperm between the mock versus day of IVF samples. (90±1.1 vs 76.0±12.8%) respectively

Conclusions: The data shows conclusively that the mock semen preparation for IVF is quite predictive of the specimen collected on the actual day of IVF. The difference in survival of sperm on the day of IVF could be due to the presence of the oocyte or other factors, and studies are currently under way to investigate this phenomenon. The mock can be used effectively as a predictive tool for troubleshooting and in assisting with making the necessary adjustments and modifications in order to maximize the outcome of the procedure on the actual day of IVF.
P3/4 – 115
PURESPERM IS SUPERIOR TO ISOLATE DENSITY GRADIENT FOR SPERM PROCESSING.
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The ideal semen processing technique should result in the selective recovery of good quality motile sperm while maintaining a high total yield of recovered sperm. It has been demonstrated that the use of density gradient media for the isolation of highly motile sperm gives improved fertilizing ability and longevity in assisted reproductive procedures. The objective of our study was to test the efficacy of two commercially available density gradient media (PureSperm – a newly introduced product, Nidacon International, Gothenburg, Sweden, and ISolate, Irvine Scientific, Santa Ana, CA, USA) used for assisted reproduction. These 2 gradients are Hapes buffered, isotonic, colloidal silica suspensions in 2 densities. PureSperm is: 40% - 80% and ISolate is: 50% - 90%, gradient. We compared the pre and post wash sperm characteristics [count, motility, recovery rate, morphology, and reactive oxygen species (ROS) levels] of the sperm fractions obtained after processing 13 semen specimens with the two media. Semen specimens processed by PureSperm gave higher recovery of total sperm count (p = 0.001), and total motile sperm count (p = 0.002) than ISolate. The recovery rate for PureSperm was 37% higher than ISolate (p = 0.006). Motility and ROS levels were similar in the two fractions (p = 0.96 and 0.38, respectively). ROS production was insignificant in the 2 mature sperm fractions recovered after a 20 minute centrifugation step involved in the gradient separation indicating a lack of oxidative stress. Sperm fractions from both gradients showed a high percentage of normal sperm forms by both WHO and Kruger’s methods. We recommend the use of PureSperm for ART purposes as higher rates of recovery of mature motile sperm in specimens processed for ART is associated with higher fertilization and pregnancy rates. PureSperm not only gives better recovery but is also cost effective (ISolate is 42% more expensive than PureSperm in the United States).

P3/4 – 116
NOVEL SEMEN QUALITY SCORES CAN PREDICT PREGNANCY IN PATIENTS WITH MALE FACTOR INFERTILITY UNDERGOING INTRAUTERINE INSEMINATION.

Individual semen parameters are ineffective in predicting pregnancy outcome in patients with male factor infertility undergoing super- ovulation/intravaginal insemination (SO/IUI). Using principal component analysis, semen characteristics can be reduced into 2 scores: Semen Quality (SQ) and Relative Quality (RQ). SQ utilizes concentration, motility, WHO and Kruger morphology, curvilinear velocity, straight-line velocity, linearity, and amplitude of lateral head displacement into a single score of overall semen quality and quantity. It gives greatest weight to sperm concentration. RQ represents the quality of motility, morphology, and motion parameters after adjusting for concentration. Our objective was to determine if these 2 scores can be used effectively in predicting pregnancy in patients undergoing SO/IUI for male factor infertility. We reduced 8 semen characteristics into 2 semen scores. Both scores are scaled to a mean of 100, SD of 10. We applied these semen scores to male factor patients undergoing SO/IUI. Pre- and post-wash semen analysis results from 192 SO/IUI cycles of 93 male factor patients were used to calculate SQ & RQ scores. Repeated measures GEE logistic regression was used to evaluate their relationship with pregnancy. Of the 192 cycles, 27 (14%) resulted in pregnancy. The increased likelihood of pregnancy was related to increased RQ (p<0.001) and SQ (p<0.002) using the post-wash semen scores. Using post-wash scores, only RQ (p<0.001) was related to outcome. Of the SO/IUI cycles in which post-wash RQ was >125, 40% resulted in pregnancy, opposed to 5% of cycles with RQ <125. Prewash SQ & RQ are positively correlated with pregnancy in patients undergoing SO/IUI. Postwash RQ scores >125 appear to be more important in predicting pregnancy in SO/IUI setting. These novel scores provide quick, simple, and reliable tools in predicting pregnancy in patients undergoing SO/IUI for male factor infertility.

P3/4 – 117
NOVEL MECHANISM OF SPERM PROTECTION BY EGG-YOLK.
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Bovine seminal plasma contains a family of proteins designated BSP-A1/A2, BSP-A3 and BSP-30-kDa (collectively called BSP proteins). These proteins are secretory products of seminal vesicles and they bind to sperm surface at ejaculation. The BSP proteins induce cholesterol and phospholipid efflux from sperm membranes indicating their role in sperm membrane modification that occurs during capacitation. Since the lipid efflux is time and concentration dependent, continuous exposure of sperm to BSP proteins (seminal plasma, SP) is detrimental to the membrane. Egg-yolk (EY) is routinely used in extenders to preserve sperm. In this report, we show that the BSP proteins bind to the low-density fraction (LDF), a lipoprotein component of the EY extender. The LDF was isolated by ultracentrifugation. The binding of BSP protein s to LDF was investigated by ultracentrifugation, agarose- gel electrophoresis, gel filtration and immunoblotting. These techniques indicated that the BSP proteins bind to LDF. In addition, LDF-BSP proteins complex could be isolated by ultracentrifugation from EY extended frozen-thawed semen indicating the high stability of the complex. Furthermore, a mole of LDF binds an average of 243-555 moles of BSP proteins indicating a high binding capacity of LDF for BSP proteins. Since EY is extensively used in preservation of mammalian sperm and that the BSP proteins destabilize sperm membranes, the binding of BSP proteins to LDF prevents their detrimental effect and this interaction appears to be crucial for sperm storage. Thus, we propose sequestration of BSP proteins of SP by LDF is the major mechanism of sperm protection by EY.

P3/4 – 118
INHIBINS, ACTIVINS AND FOLLISTATINS IN PROSTATE FUNCTION.
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Conadal inhibins and activins are major regulators of FSH, but we have identified a role for these growth factors in the progression from benign to malignant prostate disease, based on their pluriportent effects and homology to TGFβ. Non-malignant prostate tissues express inhibins and activins but in high-grade prostate cancer, the inhibin α subunit expression is lost and is associated with LOH at the 2q33 chromosome locus and with hypermethylation of the promoter of the a subunit gene. In prostate cancer, activin synthesis is unopposed by inhibin production and activins are growth inhibitory, inducing apoptosis and changes in cell morphology of LnCaP cells. The androgen independent PC3 cell line is resistant to the activin ligands. Thus, progression from androgen dependent to androgen independent growth is associated with acquisition of resistance to activin A. Resistance to TGFβ ligands is a key event in progression to malignancy. Mechanisms of inducing resistance to activins include: 1) the differential expression of follistatin (FS) activins binding proteins. PC3 cells express membrane bound FS 288 and neutralization of FS288 renders them sensitive to activin A. 2) formation of new activins with activin bC subunit and the other b subunits. A biological role for activin bC is unknown, but a change in the relative levels of the bC and bA subunit expression during cancer progression would alter the proportion of homodimers and heterodimers and reduce the levels of bioactive activin A. Thus the altered expression of bC may regulate growth inhibition by activins A. These results demonstrate new mechanisms to modify activin synthesis and action, that can contribute to malignant disease in the prostate, as well as in other reproductive tissues such as testis, breast and ovary.
Abstracts – Poster Session 3/4

**P3/4 – 119**

**DIFFERENTIAL EXPRESSION OF OXYTOCIN AND ITS RECEPTOR IN PROSTATE TISSUE FROM PATIENTS WITH BENIGN OR MALIGNANT PROSTATE DISEASE.**

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Oxytocin is expressed in the human prostate where it is thought to be involved in androgen regulation. Disruption of this regulation is implicated in the pathophysiology of benign prostatic hyperplasia (BPH). This study compares the sites of oxytocin synthesis and receptor localisation in tissue from patients with BPH and prostate carcinoma (CaP). Sections of Bouins fixed prostate tissue underwent high temperature antigen retrieval prior to immunolocalisation of oxytocin (antiserum R16, 1:100) oxytocin associated neurophysin (antiserum hNP1, 1:100), and oxytocin receptor (antiserum O20, 1:50). Oxytocin and its neurophysin were localised to the epithelial and basal cells of acini in all tissues. The intensity of the staining for both peptides was much greater in BPH tissue than in normal tissue. In contrast staining was much reduced in CaP, consistent with previous reports that local oxytocin concentrations are reduced in tissue from patients with CaP. Oxytocin receptor was localised to both the epithelial and basal cells of the glandular tissue in addition to stromal tissue. Intensity of staining in epithelial cells was increased in BPH tissue whilst almost absent in CaP. These results demonstrate very different expression of oxytocin and its receptor in benign and malignant prostate disease. Furthermore, the loss of oxytocin receptor may be important in the development of CaP.

**P3/4 – 120**

**GAP JUNCTIONS IN HUMAN PROSTATE DISEASE.**

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Gap-junctions (GJ), composed of connexin (Cx) proteins, play an essential role in cell-cell communication (GJIC) and differentiation. Disregulation of Cx expression is believed to play a role in carcinogenesis. The human prostate has been reported to express both Cx32 and Cx43; however, the expression pattern in prostate cancer (CaP) has been controversial while Cx expression in BPH has not been reported. To understand their potential involvement in prostate disease, we evaluated Cx32 and Cx43 expression in a series of human PCA (n=40), BPH (n=43) and normal prostate (n=23) specimens by immunocytochemistry. In normal prostate, punctate Cx43 stain localized to basal epithelial cells whereas Cx32 localized to luminal cells. Cx32 and Cx43 were present in 87% and 69% of normal cases, respectively. In BPH, Cx immunostain increased in epithelial cells (93% Cx43 positive; 81% Cx32 positive) and, for the first time, Cx43 was observed in periacinar stromal cells. In contrast, Cxs were decreased in PCA specimens: 65% and 38% were negative for Cx43 and Cx32, respectively with 28% negative for both. In poorly differentiated PCAs, Cx 43 and Cx32 were 10% and 40% positive, respectively, at low stain intensity. We conclude that in the normal human prostate, basal cells communicate via Cx43-GJ whereas luminal cells communicate via Cx32-GJ. In BPH, GJIC is increased in both epithelial and stromal cells which may play a role in BPH pathogenesis. In PCA, GJIC is reduced as indicated by a decreased expression of both Cx43 and Cx32 with severe loss in poorly differentiated PCAs. These alterations in Cx expression may play a role in dedifferentiation and tumor progression.

**P3/4 – 121**

**CLONING OF A NOVEL ANDROGEN-REPRESSIBLE GENE EXPRESSED IN THE MOUSE PROSTATE.**

J Singh*, L Young1, DJ Handelsman2 and Q Dong1, Dept of Medicine, University of Sydney, Sydney NSW 2006; ANZAC Research Institute, Concord Hospital, Sydney NSW 2139, Australia.

Prostate cancer deaths are due to functional escape of prostate cancer cells from their original androgen-dependent growth. To better understand the origin and evolution of hormone-refractory prostate cancer, it is important to identify and characterize genes expressed in the androgen-deprived prostate. Using suppression subtractive hybridization between the congenitally androgen-deficient (hpd) and androgen-replaced hpg mouse prostates, we have cloned a novel androgen down-regulated mouse prostate (ADMP) gene. By RT-PCR, the ADMP gene is strongly expressed in hpg mouse prostate, weakly expressed in mature castrated mouse prostate, and not expressed in normal intact or androgen-replaced hpg mouse prostates. The mRNA sequence of ADMP consists of 880 nucleotides with an open reading frame (ORF) of 231 bp that encodes for a protein of 76 amino acids. The ORF of ADMP bears 85.7% homology to the ORF of a human EST. The predicted human protein of 231 amino acids shows no secretory or nuclear localization signal but shares sequence similarity with the yeast calcium-transporting ATPase 8 (SwissProt_SpTREM11, Q12674). Further studies on the human homolog of ADMP may provide valuable insights into its role in the pathophysiology of the human prostate.

**P3/4 – 122**

**MOMENTARY INCREASE OF PLASMA CALCITONIN GENE-RELATED PEPTIDE IS INVOLVED IN HOT FLUSHES IN MEN TREATED WITH CASTRATION FOR CARCINOMA OF THE PROSTATE.**

A-C Spetz*, Division of Obstetrics and Gynaecology, B Pettersson*, E V arenhart*, Division of Urology, E Theodorsson*, Division of Neurochemistry, L-H Thorell*, Division of Psychiatry and M Hammar*, Division of Obstetrics and Gynaecology; Faculty of Health Sciences, University hospital, Linkoping, Sweden.

Background: Hot flushes occur and usually persist for many years in a majority of men treated with castration due to carcinoma of the prostate. The mechanisms behind these symptoms are unknown in men. In women the vasodilatory neuropeptides Calcitonin Gene-Related Peptide (CGRP) and Neuropeptide Y (NPY) seem to be involved. The aim of this study was to assess if the plasma concentrations of CGRP and NPY change during hot flushes in men after castration. Methods: Ten men (age 61-81 years), castrated due to cancer of the prostate and suffering from frequent hot flushes were investigated for changes in concentrations of CGRP and NPY in plasma during one day in the outpatient clinic. At least 5 samples were taken between flushes and four samples were taken during each flush. The samples were analysed for CGRP and NPY using radioimmunoassay techniques. 10 hot flushes were objectively recorded by measurements of peripheral skin temperature and skin conductance. Findings: The plasma concentrations of CGRP increased significantly (p=0.028), with 46% (95% CI; 21%-71%) during the flushes in the six men with analysable plasma concentrations. The concentrations of NPY were below detection limits. Skin conductance and skin temperature increased significantly during the flushes. Interpretation: CGRP is involved in the mechanisms of hot flushes in men castrated due to prostate carcinoma. It may thus be a similar mechanism behind hot flushes in women and in men deprived of sex steroids.
THE GLANDULAR KALLIKREIN 2 OF THE COTTON-TOP TAMARIN IS A PSEUDOGENE.
A Yvonne M Olsson* and Ale Lundwall*, Department of Clinical Chemistry, Lund University, Malmö University Hospital, Sweden.

Humans have three highly conserved glandular kallikreins; tissue kallikrein (hK1, geneKLK1), human glandular kallikrein 2 (hK2, gene; KLK2) and prostate specific antigen (PSA/hK3, gene; KLK3). PSA and hK2 are mainly expressed in the prostate and are known to be involved in the liquefaction of semen by degradation of semenogelin I and II, the gelforming proteins in semen. The biological consequence of this is still to be unravelled. PSA is a well-established diagnostic and prognostic marker for prostate cancer. It has also been found that combined measurement of PSA and hK2 enhances the discrimination between benign prostate hyperplasia and prostate cancer. We have cloned and determined the nucleotide sequence of the KLK2 orthologue in the New World monkey cotton-top tamarin. It is divided into 5 exons and the boundaries between them are at the same position as other known glandular kallikreins. It displays 90% similarity with the human KLK2, 80% with the human KLK3 and 70% with the human KLK1. The deduced primary structure suggests a 236 aminoacids long protein. Comparative analysis shows that the tamarin signal peptide is 6 as longer than the human orthologue, and the protein terminates 12 as upstream of the human C-terminal. Furthermore the catalytical triad is disturbed, HisG1, AspG6 are conserved but SerG15 is mutated to a threonine, and the protein can consequently not function as a pro tease. This suggests that the cotton-top tamarin KLK2 is a pseudogene. The gene may be non-functional due to a 15-nucleotide deletion found in an androgen responsive element upstream of the gene. Further knowledge of this could be beneficial both for reproduction and prostate cancer studies.

DIFFERENTIAL RESPONSE OF ANDROGEN-SENSITIVE AND ANDROGEN-REFRACTORY PROSTATE CANCER CELLS TO INDUCTION OF APOPTOSIS BY TRAIL.
MA Eid* and MV Kumar. Section of Urology, Medical College of Georgia, Augusta, GA.

To identify drugs inducing apoptosis in androgen-sensitive and androgen-refractory prostate cancer, the efficacy of TRAIL, a recently identified inducer of apoptosis, was investigated. Androgen-responsive (PC3AR) and androgen-refractory (PC3Neo) prostate cancer cells were treated with increasing concentrations of TRAIL protein (200ng/ml, 400ng/ml and 600ng/ml) for different periods (2hr, 4hr, 6hr, 8hr, 16hr, 20hr and 24hr). MTT assays indicated that incubation with TRAIL induced dose- and time-dependent death in prostate cells. PC3AR cells were more sensitive to TRAIL compared to PC3Neo cells. Although changes in response of the two cell lines were observed as early as 4hr with 200ng/ml of TRAIL, highly significant differences in response were observed by 8hr. Analysis of protein suggests that the resistance of PC3Neo to apoptosis could be due to increased expression of decoy receptor 2 (Dcr2) compared to PC3AR. Death receptor 5 increased in TRAIL-treated PC3AR cells, but no significant changes were noted in PC3Neo cells. This increase offers another reason for the increased death of PC3AR cells. Expression of death receptor 4 did not change in PC3Neo, but increased in TRAIL-treated PC3AR cells. Activation of TRAIL pathway was confirmed by the activation of caspase 8 and Bid in TRAIL-treated cells. Their activation was significantly higher in PC3AR cells, which suggests sustained activation of TRAIL pathway. These changes may account for the differential response of androgen-sensitive and androgen-refractory prostate cancer cells to induction of apoptosis by TRAIL through differential activation of key members of apoptotic pathway. Significance of these results and involvement of other regulatory proteins will be discussed.

INTERMITTENT ANDROGEN SUPPRESSION (IAS) IN THE TREATMENT OF PROSTATE CANCER.
N Bruchovsky, Department of Cancer Endocrinology, BC Cancer Agency, 600 West 10th Avenue, Vancouver, BC, V5Z 4E6
IAS, which takes advantage of the reversible features of LHRH agonists and antiandrogens, is a prototype therapeutic initiative that attempts to bring about long-term control of prostate cancer while minimizing the psychological and possibly biologic side effects associated with once-only castration. Since the original report of Akakura et al. (Cancer 1993;71:2782-2790) describing the feasibility of this approach, a number of Phase II trials have been performed, one of these being a multicenter prospective trial started in Canada in 1995 for the management of patients in biochemical relapse after failure of radiation for localized prostate cancer. An interim analysis of the results of treatment has yielded several observations on the relationships between baseline serum PSA, nadir serum PSA, Gleason score and time off-treatment. In a typical androgen-dependent tumour, the response of serum PSA to androgen withdrawal is biphasic but with early tumour progression, plateauing of serum PSA is observed. Ligand-independent activation of the androgen receptor, a mechanism subserving the initiation of androgen independence, can be counteracted experimentally with decoy molecules and clinically with non-steroidal antiandrogens. Serial measurements of serum PSA indicate that intermittent androgen suppression engenders a more diverse range of hormone-related responses than previously appreciated. These include: 1) repeated differentiation of tumour with recovery of apoptotic potential, 2) inhibition of tumour growth by rapid restoration of serum testosterone, and 3) restraint of tumour growth by subnormal levels of serum testosterone. The dominant type of response is an aspect of regulation that should be taken into account when planning long-term IAS of prostate cancer.
P3/4 – 127

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION FROM NEUROENDOCRINE (NE) CELLS IS ASSOCIATED WITH INVASIVE PHENOTYPE AND PATHOLOGIC STAGE RATHER THAN MICROVESSEL DENSITY (MVD) IN PROSTATE CANCER (PCA).
Sero Anandion, Vihn Dam*, Louis Begin**, Simon Chevalier*, Arnen G. Aprikian** McGill Urologic Oncology Research Laboratory, Montreal, Canada

INTRODUCTION: It has been shown that (1) NE cells in PCA express VEGF, (2) MVD increases in PCA, and (3) VEGF activates motility-promoting proteins such as FAK in human PCA cells which contain VEGF receptors in vitro. Our objective was to determine the relationship of VEGF-expressing NE cells and (1) MVD, (2) pathologic stage, (3) pre-operative hormone therapy, and (4) clinical status in human PCA. METHODS: Expression of VEGF-A and FVIII (von willibrand factor) was examined by immunohistochemistry on whole mount radical prostatectomy specimens from 61 consecutive patients with clinically localized PCA. NE cells and MVD were counted in areas of benign, high-grade PIN and invasive cancer within the same section. RESULTS: VEGF expression was found preferentially in prostatic NE cells. MVD increased significantly from benign to PIN to cancer (p<0.01). VEGF-positive NE cells also increased significantly from benign to PIN to cancer (p<0.01). However, there was no significant correlation between VEGF expression and MVD within the same regions (R values between 0.11 and 0.33). VEGF-expressing NE cells correlated significantly with higher pathologic stage (p3) (p<0.01). Pre-operative hormone therapy increased VEGF expression (p=0.06). After a median follow up of 56.8 months, patients alive with disease had higher VEGF-positive NE cells than patients with no evidence of disease (p=0.05). CONCLUSIONS: The degree of VEGF-expressing NE cells in PCA correlates with invasive phenotype, higher pathologic stage and clinical status but not with MVD. This suggests that VEGF from NE cells may be involved in progression of PCA.

P3/4 – 128

SOMATOSTATIN RECEPTOR mRNA IN SITU HYBRIDISATION IN HUMAN PROSTATE AND PROSTATE CANCER.
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INTRODUCTION: Human tumours often upregulate the expression of receptors for the antiproliferative peptide somatostatin. It is the objective of this study to investigate the presence and distribution of somatostatin receptor (SSTR1-5) subtype specific expression in normal, hyperplastic (BPH) and neoplastic prostatic tissues (CaP).
METHODS: Kryo and paraffin sections from radical prostatectomy or TUR-P specimens were used to detect and localise the gene expression of SSTR2 and SSTR4 subtypes by in situ hybridisation. Selective and well characterised non-isotopic ribonucleic acid probes were used for high sensitivity and high cellular resolution.

RESULTS: An intense SSTR2-chromogenic reaction was seen in fibroblasts, endothelial- and smooth muscle cells of the stroma. Epithelial cells also stained for SSTR2 mRNA, but giving a much weaker signal. In epithelial cells of BPH a weak staining was seen, whereas PIN and CaP showed a stronger staining reaction. SSTR subtype 4 probes hybridised stronger to epithelial cells when a comparison was made with sections stained for subtype 2. Malignant epithelial cells also showed stronger SSTR4 signals than did BPH. The stromal compartment was either negative or weakly positive for SSTR4 mRNA.

CONCLUSIONS: Our study showed upregulated expression of SSTRs in adenocarcinoma of the prostate. These results suggest that malignant epithelial cells of the prostate is a potential target for somatostatin analogues such as the SSTR2 preferring agonist octreotide, but also that novel somatostatin analogues targeting SSTR4 may be effective in the treatment of prostate cancer.

P3/4 – 129

BIOLOGICALLY ACTIVE PAC1 RECEPTOR ISOFORMS IN HUMAN NEOPLASTIC PROSTATE.

PACAP is a member of the VIP family of peptides and exists in two forms: one of 38-aa residues (PACAP-38) and a shorter one (PACAP-27). PACAPs exert their biological action by interacting with both PACAP-selective type I (PAC1) and type II (VPAC1) receptors which bind both PACAP and VIP. In the present study we demonstrate that human neoplastic prostate tissue and androgen dependent and independent (Lncap and PC3) cell lines express the hPAC1 gene and its isoforms. In Lncap cell line, PACAPs are also biologically active. The RNA extracted from above mentioned cell lines and from the tissues of patients affected with prostatic carcinoma, has been analyzed with a RT-nested PCR, using primers flanking the intersection sequence (SV1/SV2) box in the human PAC1 gene. During the first PCR round, primers designed to amplify the region spanning 872-1626 of PAC1 cDNA gave the expected product of 754 bp. This product has been amplified once more using a different set of primers, spanning the region 1200-1390 of PAC1 DNA. The studied cell lines and human neoplastic tissue express the same pattern of isoforms as demonstrated by the predicted products of 190 bp, 274 bp and 352 bp related to the null, the SV1/SV2 and SV3 variants of the PAC1 gene. In order to study if prostate cancer cells have functional PAC1 receptor, we evaluated the effects of PACAP-38 and PACAP-27 on PSA secretion by Lncap. The cells were incubated for different times (20 min, 1h, 3h, 5h and 24 h) with 10-8M PACAPs at 37°C, and the PSA in the media was assayed by ECLIA. At this concentration the effect on PSA accumulation is highest at 1 hour for PACAP 1-38 (18.15 +/- 0.2 ng PSA/mg protein).

P3/4 – 130

APOTOPSIS INDUCTION IN PROSTATIC EPITHELIAL CELLS BY ZINC IONS IN VITRO.
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Prostatic epithelial cells contain the highest levels of zinc among all organs and tissues in the human body. Zinc is accumulated primarily in the mitochondria, where it is responsible for inhibition of mitochondrial aconitate activity, thereby increasing citrate production. The present study was designed to clarify the role of zinc for human prostate epithelial cell growth and apoptosis. Human prostatic primary epithelial cells, derived from prostatic explants, were exposed in vitro to ZnCl2. Apoptosis of the cells was analyzed by determination of phospholipid membrane asymmetry, nuclear fragmentation, DNA strand breaks, changes of mitochondrial potential and cellular pro/anti-apoptotic proteins. Annexin-V/Propidium-iodide staining showed a phosphatidylinerin flip after one hour of incubation with 1 μM of ZnCl2. Western blots revealed that p53 was not involved in the apoptotic process. Bcl-2, however, was downregulated steadily in the course of apoptosis whereas Bax remained unaltered. This shift is very likely the reason for the loss of the mitochondrial potential which was detected by JC-1 staining. Compared to UV-irradiation, prostate cells exposed to 1 μM ZnCl2 did not show such a strong DNA fragmentation after 24 hours (38.6 % versus 12.6 %). Significant fragmentation of G2/M-phase nuclei and those in G0/1-phase was observed after 48 hours, where 44.5% of the nuclei stained in the sub G0/1 area (UV-irradiation: 41.7%). In summary, physiological concentrations of Zn2+ induced apoptosis without involvement of p53 by decreasing mitochondrial transmembrane potential and Bcl-2 protein levels in proliferating human prostatic epithelial cells.
P3/4 – 131
GREEN TEA AND CARCINOGENESIS
Colm Morrissey*, Matt Brush*, Adam Buser*, Jacinta O’Sullivan*, John Scolaro* and Martin Tenniswood, Department of Biological Sciences, University of Notre Dame, IN 46556
In 1998-1999, 54,000 new cases of prostate cancer were diagnosed and 39,200 men died from prostate cancer in the United States. Although genetic and hormonal factors are important, there is evidence to suggest that extrinsic factors play a role in prostate cancer incidence. A number of epidemiological studies have suggested that consumption of green tea reduces the risk of prostate cancer. Using the well established Lobund-Wistar autochthonous model of urogenital cancer, we treated Lobund-Wistar rats for periods up to 26 months with a purified decaffeinated green tea extract. We found a 50% reduction in the incidence of primary tumors of the genitourinary tract in treated animals when compared to an age-matched cohort receiving water. These data suggest that consumption of green tea affects the incidence of genitourinary tract tumors in this model of urogenital cancer. Epigallocatechin-3-gallate (EGCG) is the major catechin present in green tea and is a potent anti-oxidant. The genetic and cellular damage by oxygen free radicals can be averted by potent antioxidants like EGCG, reducing the cumulative genetic damage to the cell and preventing the accumulation of neoplastic cells. The NRP-152 and NRP-154 cell lines are derived from the dorsolateral prostate of the Lobund-Wistar rat. To elucidate the molecular mechanism of EGCG, we have treated the NRP-152 and NRP-154 cell lines with EGCG. This treatment has no apparent effect on the NRP-152 cell line, but results in reduced cell numbers and apoptotic cell death in the NRP-154 cell line. We are currently assessing anti-oxidant enzymes involved in oxidative stress and lipid peroxidation in both cell lines. This study provides a definitive scientific evaluation of the effects of green tea and EGCG and may validate their use as a natural chemopreventive agent for prostate cancer prevention. (Supported by the Coleman Foundation)

P3/4 – 132
THE MECHANISM OF CASODEX INDUCED CELL DEATH.
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Androgens such as flutamide and Casodex (bicalutamide) are designed to treat advanced stage prostate cancer by interfering with normal androgen receptor mediated processes that ensure cell survival. Long-term treatment with anti-androgens, however, leads to hormone-resistant tumors and a more aggressive phenotype. To understand the mechanism leading to hormone resistance and metastatic progression, we have treated hormone-dependent, non-metastatic LNCaP human prostate cells with 10-100μM Casodex. These treatments induce cell death in 20-60% of the cells in 48h. However, they also induce a small, yet clinically significant proportion of cells to progress to an invasive phenotype. Treatment with TNF-α induces cell death, DNA fragmentation and oligonucleosome formation, unlike Casodex, which does not induce oligonucleosomal laddering under the same conditions. Furthermore, CAD, the endonuclease responsible for DNA laddering, is down regulated in LNCaP cells by Casodex and is not detectable in a Casodex-resistant LNCaP invasive subtype, suggesting that DNA laddering is compromised in these cells. This is accompanied by sustained mitochondrial activity and decreased cell adhesiveness. Over-expression of bcl-2 in LNCaP cells attenuates the induction of cell death by TNF-α but not Casodex, suggesting that Casodex induces LNCaP cell death without depolarizing the mitochondrial membrane. Taken together, these data demonstrate that Casodex induces cell death in an indirect and incomplete fashion which results in an extended lag phase of cell survival that can be characterized by the loss of adhesiveness, sustained mitochondrial activity, decreased CAD expression and increased expression of genes that favors the acquisition of invasiveness. This suggests that the invasive phenotype induced by Casodex may be due to the temporal nature of Casodex-induced cell death and raises questions about the use of anti-androgen in a chemopreventive setting. (Supported by a research grant from the Coleman Foundation.)

P3/4 – 133
THE EFFECT OF THE ANTI-ANDROGEN CASODEX ON NRP-152 AND NRP-154 CELL LINES
Mark Brown**, Colm Morrissey*, Matt Brush*, Adam Buser*, Nikki Okezie* and Martin Tenniswood, Department of Biological Sciences, University of Notre Dame, IN 46556 and The Walther Cancer Center, University of Notre Dame, IN 46556.
Bicalutamide (Casodex) is a nonsteroidal anti-androgen indicated for use in combination therapy with a luteinizing hormone-releasing hormone analog (LHRH-A) for the treatment of Stage D2 metastatic carcinoma of the prostate. The NRP-152 and NRP-154 cell lines are derived from the dorsolateral prostate of the Lobund-Wistar rat. RT-PCR, Western analysis and immunofluorescence confirm that the NRP-152 cell line expresses the androgen receptor in vitro, however the NRP-154 cell line does not. NRP-152 and NRP-154 cell lines were treated with varying doses of Casodex ranging from 1 μM to 40 μM in a serum free media for up to 72 h. Using crystal violet assays, a dose dependent reduction in cell number for both the NRP-152 and NRP-154 cell lines was observed with a maximal loss of 55% using 40 μM Casodex after 72 h of treatment. Flow cytometric analysis using Apo-BrdU demonstrates that Casodex treatment induces DNA fragmentation indicative of late stage apoptotic cell death in both cell lines. These data suggest that Casodex can act in an androgen receptor independent fashion in vitro. Additionally, cytometric cell cycle analysis using Propidium Iodide indicates arrest in the G2/M phase of NRP-154 cells (24%) as compared to the control (12%), which is not observed in the NRP-152 cell line after treatment with 40 μM Casodex. Treating both cell lines with murine and human TNF-α (10 ng/mL) had no effect on cell number. We are currently determining the apoptotic mechanism of cell death in both cell lines. These data may provide information regarding the androgen receptor independent events triggered by Casodex that induce apoptotic cell death and explain some of the side effects that are associated with Casodex treatment. (Supported by the Coleman Foundation)

P3/4 – 134
ANDROGENS INTERFERES WITH EGF RECEPTOR-MEDIATED SIGNAL TRANSDUCTION IN PROSTATE CANCER CELLS.
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We have previously demonstrated regulation of a6b4 integrin expression and invasion of prostate carcinoma cells by androgens. In particular, transfection with an androgen receptor (AR) vector of androgen-independent carcinoma PC3 cells suppressed EGF-mediated invasion and reduces adhesion to laminin by decreasing a6b4 integrin expression. Treatment with the synthetic androgen R1881 further reduced invasion without modifying a6b4 surface expression, suggesting an interference with signal transduction mechanisms. It is known that a6b4 co-localize with EGF receptor (EGFR) in response to EGF. We have thus investigated if androgen disrupts this phenomenon in PC3-AR respect to mock-transfected (PC3-Neo). By confocal immunofluorescence we show that EGF treatment induces co-localization of a6b4 and EGFR on cell surface in both cell lines, although co-localization of the two proteins was lower in PC3-AR cells. Androgen treatment suppresses the process in PC3-AR but not PC3-Neo cells. To confirm the presence of a physical interaction between the EGFR and a6b4, EGFR and b4 were immunoprecipitated from PC3-Neo cells and the presence of EGFR revealed by Western blot. The receptor was present in both immunoprecipitates, indicating a physical interaction between the two molecules. We have also studied the localization of the androgen receptor in PC3-AR cells by confocal immunofluorescence and demonstrated a localization both in the nucleus and in the cytoplasm. In the cytoplasm, a co-localization between the EGFR and AR was present. Immunoprecipitation studies to show a physical interaction between AR and EGFR are in progress. Our data indicated that the lower invasion of PC3-AR cells is due to an AR-mediated disruption of EGF-a6b4 interaction on the surface.

 VIIth International Congress of Andrology | 151
Abstracts – Poster Session 3/4

P3/4 – 135

THE QUANTITY INDICATORS MRI OF PROSTATE CANCER.

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INTRODUCTION & OBJECTIVES: the purpose of our study was to review quantity indicators of MRI in the diagnostic of prostate cancer. It is known that majorities of pathological processes in a prostate have not any characteristic MR images and the spectrometer possibilities of the method use insufficiently.

MATERIAL & METHODS: we examined 27 patients with verified prostate cancer (T3-A, 25 with BPI I, 22 with prostate, 20 the healthy volunteers. All diagnoses were reconfirmed through a multifocal biopsy of a prostate. The devices used was “TOMIKON-110BMT” provided by “BRUKER” (Germany), with magnetic field intensities 0,243 Tesla. T1/T2 - weighted images in axial, frontal and lateral positions were obtained, repeated echo and delayed echo times equaled 530/60 and 1120/30 ms respectively. We defined proton density (PD) and “2-weighted time ms” (T2) in one of points central zone (CZ), peripheral zone (PZ) of a prostate and fatty tissue (FT) of the retroperitoneal space.

RESULTS: the observed results are presented as middle values. CZ PZ FT Normal: PD 394228 438918 465802 T2 60 ms 83 ms 198 ms Prostate cancer: PD 492866 558795 684169 T2 113 ms 128 ms 101 ms BPI I: PD 488694 517433 556370 T2 94 ms 102 ms 120 ms Prostatitis: PD 467564 467004 649301 T2 71 ms 91 ms 127 ms

CONCLUSIONS: we consider that, the pathophysiological processes (an alteration, edema, proliferation) are happening in a prostate, find a reflection at a rating of quantity indicators MRI. The quantity indicators of MRI can help in the interpretation of received pictures of a prostate, specify localization and an expansion of prostate cancer. But the diagnosis of a prostate cancer had been installed only by results of morphological researches.

P3/4 – 136

COAGULOP LOOP TRANSURETHRAL RESECTION OF THE PROSTATE (CLITURP) AND WEDGE LOOP TURP (WLTURP) PROVIDE SAFE AND EFFICACIOUS OUTPATIENT TURP.

D I Lehr*, RP Kaufman, Jr.*, Division of Urology, Albany Medical Center, Albany, NY.

Introduction and Objectives: Thin loop TURP (TLTURP) is the gold standard for the surgical treatment of benign prostatic hyperplasia (BPH-I), as it provides relief of symptoms and tissue for diagnosis. Unfortunately, TLTURP can be associated with bleeding that increases patient morbidity. Transurethral electrovaporization of the prostate (TUVP) provides relief of symptoms without bleeding and with minimal patient morbidity, but does not provide tissue for diagnosis. Hence, CLITURP and WLTURP were investigated as an alternative to TLTURP and TUVP. Methods: From 1997 to 2001, one urologist (RPK) performed 22 WLTURP followed by 45 CLITURP with the Valley Lab 40 electrocautery device at settings of 300 cut, 55 coagulation. Patient charts were reviewed retrospectively. Results: The average patient age was 60years with a pre-operative AUA symptom score of 15. 93% of patients were ready for discharge in <23 hours. 90% of the patients with no pre-operative retention were catheter free at discharge. Of the 26% discharged with a catheter, 79% were catheter free in 14 days. The three patients who remained in retention, were in retention pre-operatively. The average drop in hematocrit was 2.4%, with no patient requiring a transfusion. 9% of patients were newly diagnosed with prostate cancer of which 40% (2/5) were biologically significant. One patient experienced a bulbar urethral stricture and one patient returned to the operating room for control of bleeding. Conclusions: CLITURP and WLTURP allow outpatient TURP with 90% of patients going home without a catheter. CLITURP and WLTURP offer the safety and efficacy of TUVP with the addition of tissue for patholgical diagnosis. Hence, CLITURP and WLTURP should now be considered the standard for TURP.

P3/4 – 137

ARE PROSTATIC CONDITIONS IN CHILDREN AND TEENAGERS CRITERIA OF REPRODUCTIVE INSUFFICIENCY?

Tarosin DL, Alipian AS, Koryakin MV, Ohulov AB, Harlamov SY, Kulakov KE, Zadycan SS, Buchtuev AD. Center of Human Reproduction, Pediatric Andrology Center, Moscow, Russia.

The prostate is one of the most important androgen-sensitive glands. In the past, the pathologies associated to this organ were often considered a condition typical of mature men. Currently, this view is changing as prostate pathology is encountered more often in teenagers at the onset of their sexual life. In this study, we have conducted an investigation of prostate pathologies in 12-15 year old teenagers with a history of sexual activity. Several tests for sexually transmitted diseases were conducted on these patients. In addition, the size and volume of their prostate were measured using ultrasound. Our data clearly demonstrated that infection of the prostate can be found as early as age 14. Approximately 67% of the teenagers exhibiting enlarged prostate gland acknowledged having had careless sexual encounters. Unlike in adults, the patients’ history did not reveal acute prostatitis. The presence of ureaplasma urealytica and clamidia was most commonly encountered; these were often associated with balan infections (streptococcus and staphylococcus). In conclusion, our results indicate that chronic pathologies of the prostate may have their origins during childhood and youth.

P3/4 – 138

PROSTATE CANCER SCREENING PROGRAM IN 2689 PATIENTS AND THE APPLICATION OF TRANSRECTAL ULTRASOUND.


Prostate cancer has become an alarming urological problem. Although PSA and clinical tests are the gold standard of diagnostic methods, transrectal ultrasound (TRUS) can be helpful in localising cancer lesion. TRUS is also a method qualifying patients to prostate biopsy, even if PSA and clinical examinations are negative. The aim of this study was to asses the place of TRUS in the prostate cancer screening protocol. Material: PSA and clinical examinations of the patients were performed in a group of 2688 men ranging between 50-69 years old. Patients with PSA elevated over 4.0ng/ml and/or suspected after clinical examination of prostate cancer were sent to TRUS. Results: PSA ranged between 0,1 and 108,0 ng/ml (mean 1,89ng/ml). In 189 (7,03%) patients, PSA level exceeded 4,0 ng/ml. In this group TRUS was done to assess prostate morphology and to calculate prostate volume and PSA density (PSAD) whose mean value reached 0,152 ng/ml. Within this group, 40 (22,0%) patients had PSAD elevated over 0,15ng/ml (mean 0,24ng/ml) and 18 g. needle tru-cut sextant biopsy under TRUS controlled was performed on them. Additionally 7 patients below PSAD 0,15 were sent for biopsy (3 of them suspected in clinical examination of prostate cancer, and 4 with abnormal TRUS apperance). Totally, in the group of 47 patients after biopsy of the prostate, 23 (48,9%) had a diagnosis of adenocarcinoma (G-I-II). Among them, 3 of 4 patients (6,38% of all biopert man) were suspected only in TRUS (without clinical or PSA suspicions) In conclusion, high incidence of adenocarcinoma of the prostate in man with PSAD below 0,15 ng/ml and positive TRUS, one should consider the necessity of routine use of TRUS parallel to PSA and clinical examinations.

152 | VIIth International Congress of Andrology
P3/4 - 139
A CONTROLLED CLINICAL TRIAL WITH THE TWO-BALLOON AND THREE-CHANNEL CATHETER IN THE THERAPY OF CHRONIC BACTERIAL PROSTATITIS
Huang Wei-dong*, Xu Bing*, and Huang Wenjie*, Jiayin Andrologic Hospital of Xinjiang, Urumqi, Xinjiang, P.R.China.

To investigate the efficacy of the two-balloon and three-channel catheter(TBTC Catheter) in chronic bacterial prostatitis(CBP), 103 CBP patients have received three different therapy: (1)61 cases transurethral perfusion and drainage with antibiotics by TBTC Catheter; (2)29 cases intravenously antibiotics and (3)13 cases transurethral irrigate with normal saline. The leucithin, WBC in EPS and Meares-Stamey's fractionated urine cultures pre- and post-therapy were compared. The results showed that TBTC Catheter group had an improvement rate of 81.9% and 68.9% in complaints and desity of the leucithin, respectively, and decline rate of 75.4% in WBC and efficacy rate of 91.8% in bacteriology, there was a significant statistical difference between TBTC Catheter group with intravenous group in the leucithin, WBC and bacterial cultures (P<0.05, <0.01 and <0.01, respectively). There was a significant difference between saline group with intravenous group in decline of WBC(P<0.05), but no difference in leucithin and bacterial cultures. Among 61 patients treated with TBTC catheter, there were 3 cases to occur mild allergic reaction in urethral. The paper showed that transurethral TBTC Catheter had higher clinical efficacy than traditional intravenous treatment for CBP. Authors believe that the perfusion of drug through with the TBTC Catheter may relieve inflammatory reaction in urethro-prostatic lumen by physical irrigated action and provide a relative higher local antibiotics concentration in prostatic glandular ducts system.

Key words chronic bacterial prostatitis; catheter; transurethral perfusion; therapy

P3/4 - 140
MANAGEMENT OF NEGLECTED/UNTREATED OLD CASES OF FRACTURE PENIS.
Dr. A. L. Bhat & Dr.G. Saxena Department of Urology S. P. Medical College Bikaner, Rajasthan India.

Introduction & Objective: To evaluate the modalities of management of neglected / untreated old cases of fracture penis and to see feasibility of using the inner prepuccal skin flap for replacement tunica albuginea. PATIENTS & METHODS: Eight untreated,neglected, conservatively treated but healed cases of fracture penis presented to us with choree, painful erection & / or painful coitus. One of the patient had ipsilateral fracture twice in 28 months. In three patients soft tissue X-ray showed calcification in plaque. Ultrasonography revealed thickening of tunica at the site of fibrosis & was casting acoustic shadow suggestive of calcification in three patients. Cavernooscopy showed loss of distensibility in five cases & bending of penis in two cases. In six patients the plaque was excised & the resulting gap in tunica albuginea was covered by resurfacing of tunica albuginea in 4 patients and with inner prepuccal skin flap in two patients. The patients who refused surgery were managed by tamoxifen and serapetidases. RESULTS: The Patients who underwent surgery had very good results and discharged from hospital in 5-7 days. They reported to have normal sexual intercourse in 6-8 weeks. Follow up was 6 months to 10 years & one of the patients with skin flap replacement of tunica had well defined prominence at the site of flap. The patients managed conservative ly had persistence of choree, painful erection and painful coitus. CONCLUSIONS: Surgical modality is the treatment of choice for fibrous plaque in neglected, untreated & complicated cases of fracture penis. Excision of plaque and resurfacing the tunica albuginea if the plaque is small, and in patients with larger gap inner prepuccal skin flap or penile skin flap is the treatment advised.

P3/4 - 141
DRUG INDUCED ISOLATED PENILE SKIN GANGRENE - A STUDY OF 7 CASES.
Dr. A. L. Bhat & Dr.G. Saxena Department of Urology S. P. Medical College Bikaner, Rajasthan India.

Introduction: Cutaneous drug reaction by NSAIDS & certain antibiotics are not uncommon but isolated penile skin gangrene is very rare. On reviewing the literature, there are few case reports of penile gangrene due to coumarin. We present our experience of isolated penile skin gangrene on account of rarity & unexplained pathogenesis. Patients & Methods: We managed seven cases of penile skin gangrene from Oct 1997 to December 2000.Two patients had severe oedema of penis, scrotum with blebs & discoloration of involved skin after chloroquin injections & another patient had similar reaction following ingestion of ciprofloxacin. Patients were given broad-spectrum antibiotics, cortisones & vasodilators but without much relief. Penile & scrotal skin became gangrenous in 5-7 days. Four patients had penile skin gangrene following ingestion of NSAIDS. None of the patient had any other gangrenous patch on any part of the body including mucocutaneous junction. Gangrenous skin and slough was removed when line of demarcation became clear. The slough was extending up to darts- fascia level & surprisingly the inner prepuccal skin was healthy in all the seven cases. All the patients did well in post operative period with systemic & local antibiotics and wound dressing. The raw area was covered with inner prepuccal skin & by mobilising the proximal penile/ scrotal skin after about three weeks when local wound became healthy. Discussion: Penile gangrene due to chloroquine & ciprofloxacin toxicity is rare. The underlying mechanism may be penile vasculitis or toxic epidermal necrosis which is known complication of anti coagulants. In both pathology area involved are multiple & mucocutaneous junction is usually involved but in our cases there was no evidence of extra genital skin involvement.

P3/4 - 142
INHIBIN β EXPRESSION IN HUMAN CONGENITAL TESTICULAR PATHOLOGY. A Serrano*, D Hardisson, D Val, ²P Gonzalez-Peramato, ²J M Nistal, ²J Regadera*, and ²CA Suárez-Quian*. ²Dept Pathol & Urol, Guadalaja Univ Hosp; ²Dept Pathol, La Paz Hosp; ²Dept Morphol & Pathol, Autonoma Univ Madrid, Spain; ²Dept Cell Bio, Georgetown Univ Med Cent, Washington, DC, 2000

While the immunoexpression of inhibin β (Iβ) has been used to make correct diagnoses for both testicular and ovarian Sertoli cell (SC) tumors, its usefulness as a marker for congenital testicular pathology is not known. Thus, we examined Iβ immunostaining in normal adult SC (12 cases), cryptorchid tests (CT) (43 cases), male pseudo hermaphrodites (MPH) (17 cases) and androgen insensitivity syndrome (AIS) (8 cases) and the results compared to SC maturation and degree of complete spermatogenesis. In normal testis, slight immunostaining was noted in SC cytoplasm (2+), but staining did not correlate as a function of the cycle. In the testicular pathologies examined, the results are presented in the table.

<table>
<thead>
<tr>
<th>TESTICULAR PATHOLOGY</th>
<th>GENERAL TISSUE HISTOLOGY</th>
<th>Iβ STAINING INTENSITY</th>
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<tbody>
<tr>
<td>MPH</td>
<td>Large tubules. local spermatogenesis.</td>
<td>SC (3+)</td>
</tr>
<tr>
<td>POST-PUBERTAL CT</td>
<td>Small tubules. no spermatogenesis</td>
<td>(2+)</td>
</tr>
<tr>
<td>POST-PUBERTAL CT</td>
<td>Very dysgenic tubules</td>
<td>(2+)</td>
</tr>
<tr>
<td>PRE-PUBERTAL CT</td>
<td>Immature Sertoli cells</td>
<td>SC (3+)</td>
</tr>
<tr>
<td>AIS</td>
<td>Immature tubules/SC adenomas</td>
<td>(2+)</td>
</tr>
<tr>
<td>AIS</td>
<td>Immature, atrophic tubules/dense SC</td>
<td>(2+)</td>
</tr>
</tbody>
</table>

In both the AIS and CT, tubules lacking peritubular myoid cells exhibited SC negative for Iβ. Conclusion: Inhibin β is necessary for maturation of the sex cords into mature seminiferous tubules. Inhibin β is expressed less in immature SC exhibiting dysgenesis, whereas mature SC exhibit more robust staining, even if these latter are present in either cryptorchid or AIS testes.
**Abstracts – Poster Session 3/4 & 5/6**

**P3/4 – 143**

**B CATENIN IS OVER-EXPRESSED IN HUMAN HYPERPLASTIC LEYDIG CELLS**


E-Cadherin (EC) and β-catenin (BC) participate in mechanisms of cellular adhesion, signal transduction and proliferation. Both proteins are found in Sertoli cells (SC) of different animals, but their presence in pathological testes with altered functional activity of SC and Leydig cells (LC) has not been explored. Thus, we studied the expression of EC and BC in human testis of normal (12 cases), cryptorchid (CT) (43 cases, 11 of which had LC adenomas), and 5 benign testicular tumors. EC was present in SC cytoplasm, near sites of cell adhesion, but not in germ cells; staining intensity diminished significantly in cases of hypospermatogenesis, being almost absent in altered SC containing lipid vacuoles. EC was also detected in normal LC, but only traces could be discerned in the cytoplasm. BC was expressed in granular form in the cytoplasm and did not become more intense near the plasma membrane. BC was present in normal SC; but was absent also from germ and peritubular cells. In normal LC, BC was present at a 1+ level in the cytoplasm, but in hyperplastic LC of CT it was present at a 2-3+ level. In addition, the hyperplastic LC appeared to express βC also within the nuclei. The presence of this intense intranuclear βC in LC, similar to what happens in mammary tumors, suggest that mutations may occur in the βC gene in hyperplastic LC and in LC adenomas that are sometimes observed in post pubertal CT and in benign LC tumors. BC mutations correlate with limited infiltration of the malignant tumors of other tissues and with good prognosis. Thus, the possible presence of these mutations in the BC genes of LC adenomas and LC tumors may help explain the low frequency of infiltration and malignancy of LC tumors in humans.

**P5/6 – 001**

**GERM CELL DEGENERATION IN MEIOTIC AND IN POST MEIOTIC ARREST OF SPERMATOGENESIS IN HUMAN TESTIS.**

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We showed that FAS mRNA and protein are expressed in scattered human germ cells which are probably undergoing apoptotic degeneration (ICEM,85,2000). Here we analysed apoptosis and its relationship with FAS expression in human germ cells. Tests biopsies from azoospermic men were included on the basis of histological findings: Normal Spermatogenesis (NO) (n=10); Meiotic Arrest (MA) (n=10); Postmeiotic Arrest (PMA) (n=10). Epon-embedded specimens were used for ultrastructural analysis and for the quantitative evaluation of apoptotic germ cells at light microscopy. Immunohistochemistry was used for FAS protein expression. Ultrastructural features of apoptosis were present in germ cells during first meiotic prophase, and in spermatids during Golgi and cap phases. Degenerating cells were eliminated by phagocytosis of Sertoli cells or by lumen shedding. The number of apoptotic germ cells per 100 Sertoli cells (APO/100Se) was higher in PMA compared to MA and to NO (p<0.0005), although some cases of MA showed a high rate of apoptotic germ cells. The same pattern was observed for the number of FAS-positive germ cells per 100 Sertoli cells (FAS+/100Se). The number of APO/100Se was highly and positively correlated with the number of FAS+/100Se (r=0.78; p<0.0000) and it was negatively correlated with the number of elongating spermatids (r=-0.60; p<0.0027), while no correlation was found between FAS+/100Se or APO/100Se and serum level of FSH. The low efficiency of spermatogenesis in cases of post meiotic arrest of maturation was associated to a high rate of apoptotic degeneration of germ cells during meiosis and early spermiogenesis, and it was correlated with FAS expression in germ cells committed to suicide.

**P5/6 – 002**

**FSH RECEPTOR ABLATION AND DELAYED SEXUAL MATURITY IN THE MALE: IMPLICATIONS FOR CONSTITUTIONAL DELAY OF PUBERTY.**

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In the complex process of mammalian spermatogenesis the development of a diploid germ cell into a haploid mature spermatozoon is orchestrated in a time frame unique for each species including man. If the various hormonal signals including environmental cues that play a critical part in initiating these events are not properly executed, various deficiencies including delay in sexual maturity or puberty are likely. In this study we have followed testicular development and first wave of spermatogenesis in the FSH receptor knockout (FORKO) mouse from 7-days post-partum. The drastic reduction in testicular weight set in at this age persisted into the adult stage in the FORKO’s. Histological and flow cytometric analysis revealed that the round spermatids were abundant on day 21 in the wild type and heterozygous males but they were few and present only in some tubules of the FORKO’s. There were no elongated spermatids in FORKO males on day 35. The sperm produced by day 49 FORKO’s were already aberrant, a feature that persisted in to adulthood in these animals. As all these changes occurred in a background of normal circulating testosterone levels we may conclude that the delay in testicular development is a consequence of the loss of FSH-R signaling. The FORKO males took 25 days longer than the wild type males to sire their first litter as determined by mating studies. Based on these data we suggest that the FORKO mouse might be a useful experimental model to define the molecular mechanisms that underlie the delay in puberty. (Study by CH-IR)

**P5/6 – 003**

**HYPOTHYROIDISM INHIBITS TESTICULAR MATURATION ON THE WAY DIFFERENT THAN ESTRADIOL DOES.**


Male rats were treated daily from 5th to 15th day of life: (1) 1% solution of 6-propyl-2-thiouracil (PTU) administered to their feeding mothers, or (2) s.c. injections of 12.5μg of estradiol benzoate (EB), or (3) PTU+EB. Controls (C) received solvents. At autopsy on 16th day blood was taken for determinations of free triiodothyronine (FT3), free thyroxine (FT4), testosterone (Te), estradiol (E), rat-FSH, rat-prolactin (PRL). In paraffin histological slides quantitation of seminiferous epithelium cells was performed.

Results: (1) After PTU hypothyroidism (H) in pups was confirmed by inhibition of T3 secretion to undetectable level and T4 to 6,5% of C. Undetectable was also PRL, while FSH, Te and E were unchanged vs C. Testicular weight was inhibited (55,9% of C), while the number of Sertoli cells increased (119% of C). H reduced differentiation of A spermatogonia into B and arrested spermatogenesis on prophase stage. However, the number of A spermatogonia increased up to 251% of C (accumulation in spite of decreased differentiation?). (2) EB reduced significantly serum FSH and Te, did not affect FT3 and FT4 and increased PRL. Testicular weight and Sertoli cell number were significantly reduced (34.3% of C). Differentiation of A spermatogonia was decreased, however, EB did not arrest premeiotic spermatogenesis. (3) After PTU+EB serum FT3 and FT4 remained lowered as after PTU alone, FSH and Te were inhibited as after EB alone. Reduced differentiation of spermatogonia and arrest of spermatogenesis were present as after PTU alone.

H inhibits testicular maturation on the way different than EB does. While the effect of H was due to reduced FT3 and FT4 secretions and not of FSH or Te, the effect of EB resulted from decreases in FSH and Te.
P5/6 – 004
ABNORMAL SPERMATOGENESIS IN DOUBLE-MUTANT MICE WITH TARGETED DELETIONS OF TNP1 AND TNP2.
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Transition proteins (TP1, TP2) are expressed in elongating spermatids and are associated with chromatin during initial condensation. To study the function of the TPs, mice with targeted deletions of the Tnp1 and Tnp2 genes, which code for TP1 and TP2 respectively, have been generated. In all mice with double mutant genotypes, spermatogenesis was complete and sperm were transported to the epididymides. The number of sonication-resistant spermatids (SRS) was normal in “triple-minus” males (TP1(-/-),TP2(-/-);TP1(-/-),TP2(+/-)) indicating progression of chromatin condensation during spermatogenesis. A significant reduction in SRS (60%) was found in the double knockout males (TP1(-/-),TP2(-/-)), possibly a result of delayed or incomplete chromatin condensation. Epididymal sperm counts from both “triple-minus” and double knockout males were significantly lower than controls (26-29% and 84%, respectively). As much as 50% of the total sperm cells isolated from the cauda were bound together by cytoplasm and enclosed within clusters by a single membrane. No males with “triple-minus” or double knockout genotypes were able to produce offspring through natural mating with wild-type females. These results indicate that in the absence of TPs chromatin condensation continues but that abnormalities in the process render released sperm functionally impaired resulting in male infertility.

P5/6 – 005
ESTABLISHMENT OF A MOUSE GERM CELL LINE EXPRESSING THE GFR α-1 MEMBRANE RECEPTOR.
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We recently established a putative germ cell line from 6-day-old mouse testes using the Simian Virus Large T antigen under the control of a Ponasterone A inducible promoter. While the cells escaped the hormonal control of Ponasterone after a finite number of generations and expressed the immortalizing gene constitutively, their growth remained slow and the cells exhibited morphological features typical of spermatogonia at the light and electron microscopic level. It has recently been shown that receptors such as c-kit, ret, and GFRa are expressed at the surface of different subtypes of spermatogonia, possibly corresponding to different stages of spermatogonial differentiation. GFRα-1 and GFRα-2 are the receptors for the ligands glial cell derived-neurotrophic factor (GDNF) and neurturin, respectively. These growth factors belong to a family of neurotrophic growth factors related to TGF-β. Binding of the ligands to GFRα receptors activates the ret-mediated signaling pathway. We have used these markers to determine the precise phenotype of the immortalized germ cell line. Only a low percentage of the cells express c-kit, while a majority express the GFRα-1 receptor, thus indicating that this putative germ cell line might derive from a spermatogonial stem cell.

P5/6 – 006
CLONING AND CHARACTERIZATION OF A HAPLOID GERM CELL SPECIFIC cDNA(β390) ENCODING A NOVEL SPERM TAIL PROTEIN FROM MOUSE TESTIS.
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Formation of the tail in the developing sperm is a complex process involving, for example, the organization of the axoneme, assembly of outer dense fibers and fibrous sheath and transport of periaxonemal proteins from the cytoplasm to the tail. Although detailed morphological description of these changes are available, the molecular mechanisms remain to be fully elucidated. We have isolated a new gene, named β 390, from a haploid germ cell specific cDNA library of mouse testis encoding a novel sperm tail protein. β 390 cDNA is 1.1kb long, carrying a 762bp ORF that predicts a 254aa protein. The deduced amino acid sequence includes six Proline-Glycine-Proline repeats which are also present in two Drosophila homologous sequences. Transcription of β 390 mRNA is exclusively observed in haploid germ cells. Antibody raised against β 390 identified a testis-specific 32KDa band in Western blotting analysis and the protein was localized in the whole length of the tail of the elongating spermatid and mature sperm. Sequence analysis of PCR-amplified DNA and southern blot results indicate the presence of β 390 related sequences in other animals, including rat and human. β 390 may therefore be an important constituent of the mammalian sperm tail.

P5/6 – 007
CHARACTERIZATION OF HAPLOID GERM CELL SPECIFIC KINASE 'HASPIN' PROMOTER AND ITS ACTIVITY DEFINED BY TRANSGENIC MOUSE EXPERIMENTATION.
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We have cloned haspin cDNA and its genomic DNA construct (1999, I.B.C. 274 p17049, 2001, Mol. Hum. Reprod in press). Haspin is a unique protein kinase, first isolated from mouse testis. Both human and mouse Haspin expressed specifically in testicular germ cells, have protein kinase activity, and are suggested to play a role in cell cycle arrest in haploid spermatids. Expression of human haspin by Northern blot analysis showed that the major transcript was 2.8 kilobases and was detected exclusively in the testis. Haspin contained a part of the consensus subdomain (I-III) of the kinase catalytic domain, leucine zipper, potential phosphorylation sites, and MEF2B homologous region. The genomic structure of haspin showed it to be intronless and the whole transcription unit was in an intron of the integrin αE2 gene. Here, we present a haspin promoter element which specifically activated transcription in the testis and not in any other organs by transgenic mouse experimentation. This promoter element contained GC-rich sequence but not the previously reported DNA sequences to bind transcription factors.
P5/6 - 008
INTRACELLULAR DISTRIBUTION OF RBM7 DURING MEIOSIS AND INTERACTION WITH RNA SPlicing FACTORS.
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We have previously cloned a putative RNA splicing factor, RNA binding motif protein 7 (RBM7) and described its mRNA expression in the tests. To examine RBM7 protein expression and intracellular localization, we obtained an affinity-purified, rabbit polyclonal antibody against a peptide corresponding to amino acid residues 203-224 of the mouse RBM7. This antibody recognized the predicted 35-kDa RBM7 protein both in testicular lysates and in in vitro translation reactions. It was thus used to probe adult rat testis sections for RBM7. We found that, consistent with our previous in situ hybridization data, RBM7 immunoreactivity closely paralleled the entry and progression of meiosis. RBM7 expression commenced in type B spermatogonia, spanned the entire spermatocyte development and extended to round and early elongated spermatids. RBM7 appeared nuclear up to mid-pachytene stage and became cytoplasmic later. The RBM7 structure, molecular phylogeny and intracellular localization implied a role in pre-mRNA processing. To explore this possibility, we used yeast two-hybrid and GST pull-down assays to show that RBM7 interacted with splicing factor 3b subunit 2 (SAP 145) and with splicing regulator SRp20. We propose that RBM7 functions in pre-mRNA processing during entry into meiosis and during specific stages of spermatocyte development.

P5/6 - 010
SERUM INHIBIN β VALUES REFLECT THE EFFICACY OF SPERMATOGENESIS IN A POPULATION OF 106 INFERTILE MEN
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The aim of the present study was the validation in our hospital of the dimeric assay for serum Inhibin β and the evaluation of its usefulness as a marker of spermato genesis. Inhibin β was assessed for 106 patients attending our infertility clinic. Usual reproductive hormones were also assessed concomitantly to routine semen analysis and, for some of the men which were azoospermic, results of testicular histology and/or of the Caryotype were obtained. We found that serum Inhibin β level was positively correlated with the level of sperm production as reflected by the sperm concentration and negatively correlated with serum FSH. Inhibin β concentrations were found to be superior to FSH concentrations for discriminating between subgroups of patients with different levels of sperm production. For example, the differences in the Inhibin β levels in severe oligozoospermia (≤5×10⁶ sperm/ml) compared to non obstructive azoospermia were more pronounced than the differences in the FSH (p=0.0002 vs p=0.007, respectively). Inhibin β levels in patients with non obstructive azoospermia were ten times lower than in patients with normal sperm concentration. Concentrations of inhibin β offered to distinguish unambiguously between obstructive and non obstructive azoospermia, which was not the case for none of the other hormones assessed. Finally, we found very low Inhibin β levels in Klinefelter cases and various levels according to testicular histology. Further studies are in progress to investigate if the assessment of Inhibin β could represent an interesting non-invasive marker of focal hypospermatogenesis in men with non obstructive azoospermia candidates for an ICSI.

P5/6 - 009
AMH AND INHIBIN β AS MARKERS OF SERTOLI CELL FUNCTION DURING A STEROID BASED HORMONAL CONTRACEPTIVE REGIME IN MEN.
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Anti-Mullerian hormone (AMH) a glycoprotein marker of immature Sertoli cell function, is produced in considerable amounts until puberty. With the advent of rising testosterone (T) levels at puberty, AMH falls to virtually undetectable levels in young adulthood. Inhibin β is also a marker of Sertoli cell function both in the immature and adult tests. Recent studies suggest that normal spermatogenic progression is required to maintain Sertoli cells in their fully differentiated state. Thus, in some infertile men with hypospermatogenesis, de-differentiation of the Sertoli cells occurs with resumption of AMH expression in the adult tests. This study examines for the first time in man, the effects of experimental reversible suppression of spermatogenesis and testicular steroidogenesis on circulating AMH levels in healthy adult men receiving a novel contraceptive regime which combined T implants with the pro-lactin inhibitor. Quinagolide (Q, Novartis). 46 healthy men were randomised to one of three treatment groups. Group A: use T 1000mg, Q 3mg daily oral pills; Group B: T 1200mg T plus oral Q 75mg/day. Group C: 800mg T plus oral Q 75mg/day. T implants maintained T levels in the high normal to supraphysiological range for 16 weeks. Q was administered for 2.4 weeks. Men were followed for 8 weeks for recovery. 40 men completed treatment. Subjects that achieved azoosperma were 5/9 (55.5%), 3/14 (21.4%) in group comparison. N.S. Corresponding sperm density fell from 55.5±9.1 to nadir of 2.4±1.1, 41.9±7.9 to 1.0±0.8, 47.1±10.2 to 13.2±3.3. Pretreatment plasma AMH levels were 65.8±10.4, 70.5±8.3, 93.9±10.3 pmol/mL for groups A, B and C respectively. Percentage change from baseline at Week 16 (sperm suppression nadir) was -0.1±13.9, -1.6±13.49, -4.2±1.67 (N.S) There was no difference between groups or between the azoospermic vs oligozoospermic responders. Inhibin β decreased significantly (p=0.05) from 178.6±21.66 to 150.6±13.87 pg/mL Group comparison NS. We have shown that spermatogenic suppression to oligozoospermia for around 16 weeks does not appear to modulate Sertoli cells function to the extent that a pre-pubertal state pertains but the fall in inhibin β confirms that there is attenuation of Sertoli cell function from gonadotrophin withdrawal but that the short duration of suppression prevented the development of changes in AMH expression.

P5/6 - 011
STIMULATION OF DNA REPAIR BY THE SPERMATIDAL TP1 PROTEIN N. Caron, S. Veilleux and G. Boissonneault*, Département de Biochimie, Université de Sherbrooke, Québec, Canada

The important change in chromatin structure that takes place during spermiogenesis in mammals is characterized by a transient increase in DNA single-strand breaks (SSB). The mammalian transition proteins 1 (TP1) is expressed at high level at mid-spermiogenesis steps coincident with the chromatin remodeling and we hypothesize that the protein could be involved in the repair of these lesions since SSB are no longer detected in terminally differentiated spermatids. Using a sensitive in vitro assay, we show that TP1 can stimulate the repair of single-strand breaks in the presence of a limiting concentration of DNA ligase. To establish whether the stimulation of SSB repair by TP1 can translate into a stimulation of DNA repair in cellulo, UV-irradiation of a reporter plasmid harboring the luciferase gene was used in order to generate pyrimidine dimers. These DNA lesions require ligase I as the ultimate step in the nucleotide excision repair process. Upon transfection of the damaged reporter plasmid into Cos-1 cells stably expressing the mouse TP1 protein a four-fold increase the reporter gene activity was observed compared to the control Cos-1 cells not expressing the TP1 protein. This latter observation suggest that the DNA repair activity in these cells is enhanced. Therefore, aside from its role in DNA compaction, this major transition protein may contribute to the yet unidentified enzymatic activity responsible for the repair of SSB at mid-spermiogenesis steps. These results also suggest that the TP1 protein have the potential to participate in the repair process following genotoxic insults and therefore may play an active role in the maintenance of the integrity of the male haploid genome during spermiogenesis.
P5/6 - 012

MULTIPLE KINESIN-RELATED MOTORS IN THE MAMMALIAN TESTIS.

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The transformation from precursor cell to spermatoozon involves drastic rearrangement of the cell's cytoskeleton with the formation and dissolution of several specific microtubule complexes. It is likely that numerous kinesin-related motor proteins play important roles in these cytoskeletal changes. Six new kinesins were identified previously in the mammalian testis; 3 of these areas are members of mitotic motor superfamilies. Motor isoforms KIFC5A-C are members of the C-terminal motor subfamily that has been shown to crosslink microtubules in the mitotic spindle. KIFC5 proteins are likewise localized to spindle structures in spermatogenic cells but are also found in the spermatic manchette and the sperm flagella. We propose that these isoforms participate in a common microtubule bundling activity in these diverse complexes in the testis. In addition to the KIFC5 group, we have identified another pair of motor isoforms found in germ cells with possible roles in spermatid maturation. These motors, called KRP3A and KRP3B, do not closely resemble any proteins already entered into the database and lack an extensive coiled-coil structure characteristic of most kinesins. Gene expression studies demonstrate that KRP3 messages do not appear until the pachytene stage of spermatocytes. Interestingly, immunolocalization of KRP3 motors indicates that they are associated with condensing spermatocyte chromosomes and spermatozoal nuclei. Characterization of molecular motors in cells of the seminiferous epithelium will provide an important insight into the role of cytoskeletal rearrangements in the numerous forms of motility displayed by male germ cells.

P5/6 - 013

CDK2β IS THE STARTER KINASE FOR MEIOTIC MPF DURING MOUSE SPERMATOGENESIS.

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Control of the mitotic cell cycle relies on the accurate coordination and activity of the cyclin dependent kinases. The role of these key regulators for the meiotic cell cycle is less well known. Recently, cyclin A1 and its kinase has been proposed to be key in the cascade that leads to MPF activation and meiotic cell division. We have identified a splice variant of the mitotic Cdk2 gene, called Cdk2β, that is highly enriched during meiosis and binds specifically to cyclin A1. To test the hypothesis that the Cdk2β/cyclin A1 kinase is the meiotic starter kinase, we have studied its expression, binding, and activity during meiosis. Our data show that Cdk2β is expressed, at both the mRNA and protein levels, exclusively during late pachytene to the end of MI of meiosis. Using enriched populations of germ cells, we also show that the somatic form of Cdk2, Cdk2α, is not found during meiosis, but is expressed only in spermatogonia and pre-leptotene cells. We further show that Cdk2β binds cyclin A1 and is active during meiosis. We conclude that Cdk2β is the starter kinase that associates with cyclin A1 and initiates the meiotic cell cycle. [Supported in part by the South Plains Foundation and the March of Dimes (#1FY-99-601) to SER and #5 T32 HD07271-16 (CAK)].

P5/6 - 014

IDENTIFICATION OF CKS2 AS A BINDING SUBUNIT OF THE MEIOTIC CDK2 ISOFORM, CDK2β.

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Cell division is controlled, in part, by cyclin/cyclin dependent kinase complexes which phosphorylate various downstream targets. Our research focuses on the alternatively spliced Cdk2 isoform, Cdk2β. Cdk2α controls the G1/S transition during mitosis, but the role of Cdk2β is unclear. Data from our lab suggest that Cdk2β may be a key player in meiosis due to its expression in prophase of meiosis I in spermatogenesis. To test the hypothesis that Cdk2β is regulated by other binding proteins during meiosis, we used the yeast two-hybrid system to identify binding partners of Cdk2β. Screening of an adult mouse testis cDNA library identified a strong Cdk2β partner as the mouse homolog of Cks2 (CDC28 kinase subunit). Cks proteins have been reported to be required for entry into mitosis, and may target the cyclin-Cdk complex to different substrates. To date, there are no data on the function of Cks proteins during meiosis. Here, our experiments show that Cks2 binds to Cdk2β more strongly than to Cdk2α. Furthermore, we have examined the expression of Cks2 by RT-PCR and in situ hybridization and show that Cks2 is a) most abundant in the adult testis, b) more abundant in the testis than Cks1, and c) is expressed primarily in germ cells. Immunohistochemistry data also show the localization of the Cks protein in the same meiotic cell types as Cdk2β. These data show for the first time, differential expression of the closely related Cks1 and Cks2 genes and suggest that Cks2 may play a role in regulation of Cdk2β during meiosis in the male mouse. [Supported by grant #1FY99-601 from the March of Dimes (SER) and #5 T32 HD07271-16 from the NICHD (ENA).]

P5/6 - 015

IMPAIRMENT OF SPERMATOGENESIS AND MAST CELL PROLIFERATION IN RATS EXPOSED TO ALCOHOL DURING PRE-PUBERTAL PERIOD.

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Ethanol intake is detrimental to cells, organ systems and physiological functions including pituitary gonadal axis. The present study evaluated the effect of ethanol exposure of prepubertal male rats on spermatogenesis and mast cell characteristics. A single oral dose of ethanol (4g/Kg. body weight) was given daily at the same time to prepubertal rats (30 days old) for 15 days to one group and for 37 days to another group. Ethanol induced significant changes in the food intake and body weight of rats in the latter group. Though no marked change was recorded in the testicular weights but a significant decrease occurred in the diameter of the seminiferous tubules in the ethanol treated rats. In addition seminiferous tubules appeared vacuolated and contained few spermatogenic cells. None of them had spermatids or spermatoozoa in their lumen till 15 days of treatment. The latter stages were seen in some of seminiferous tubules of rats treated for 37 days. At no stage spermatoozoa were observed in the epididymal fluid. Mast cells as identified by toluidine blue occurred in the tunica layer, both in control and treated rats. Their number increased significantly after ethanol treatment for 37 days and the cells appeared highly granulated. Though the exact significance of increased number of mast cells in the testes of alcohol treated rats is not known, possibly the detrimental effects of alcohol may be mediated through mast cell secretions.
P5/6 – 016

SEMINIFEROUS EPITHELIUM CYCLE LENGTH IN DONKEYS
(Equus asinus).
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The knowledge of the cycle of the seminiferous epithelium is fundamental to fully understand and utilize techniques for quantifying spermatogenesis. There is no report in the literature showing the length of seminiferous epithelium cycle in donkeys. In the present study, the duration of spermatogenesis was estimated in five sexually mature animals using intratesticular injections of H3 thymidine. Animals were castrated at 1th, 7 days, and 14 days after injections and had their testes fixed through the testicular artery with 4% glutaraldehyde. The testis tissue was embedded in plastic and routinely prepared for histological and autoradiographic analysis. The estimated duration of each spermatogenic cycle in donkeys was 10.5 ± 0.3 days. The total duration of spermatogenesis, considering that it takes approximately 4.5 cycles to be completed, was estimated at last 47.3 days. The approximate primary spermatocyte life span is 15.9 days, while spermiogenesis in donkeys lasts 15.5 days. Staging in donkeys was based on the tubular morphology system, where 8 stages of the cycle are yielded for all species. The relative stage frequencies in donkeys obtained from 400 seminiferous tubules cross-sections for each animal were as follows: stage 1, 18.1 ± 1.6%; stage 2, 2.9 ± 0.4%; stage 3, 3.8 ± 1.2%; stage 4, 19.3 ± 0.3%; stage 5, 8.9 ± 0.6%; stage 6, 22.6 ± 1.5%; stage 7, 6.3 ± 0.7%; stage 8, 9.9 ± 0.5%. The pre-meiotic, meiotic and post-meiotic phases’ relative frequencies were, respectively, 33%, 19.3% and 47.7%, being very close to the frequencies observed for stallions. However, the duration of spermatogenesis in donkeys is about 15% shorter than in stallions (12.2 days).

P5/6 – 017

MORPHOMETRIC AND HORMONAL ASSESSMENT IN MEN WITH AZOSPERMIA AND SEROTOLI CELL-ONLY SYNDROME (SCO).
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OBJECTIVE AND METHODS: Material consist of 98 patients with clinical azoospermia, out of this group 24 men were qualified into statistical analysis with SCO and permeable sperm ducts. In all patients surgical biopsy from unilateral testis was done following hormonal assessment blood serum (LH, FSH, testosterone, PRL, estradiol). Surgical specimen was evaluated hist-pat. with special attention paid to: Sertoli cells, basal membranes, medial cells, and interstitial cells. Also morphometric measurements of nuclei of Sertoli cells Leydig cells and medial cells were done. Patients with SCO were divided into two groups: SCO with normal interstitial tissue (NSCO) and SCO with hyperplasia Leydig cells (SCHO). RESULTS: Statistical analysis revealed significant differences in nucleus’s size of Sertoli cells (p<0.0102), medial cells (p<0.0175) in both groups. In NSCO group notably higher level of PRL was noticed. Non-parametric test showed the dependence between Sertoli cell’s nucleus’s size and thickness of basal membrane of the spermatogenic epithelium (SE). CONCLUSIONS: The size of nucleus of Sertoli cells is connected with thickness of basal membrane of SE and interstitial gland’s condition, which appeared after statistic analysis.

P5/6 – 018

INCREASED GERM CELL PROLIFERATION IN INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) DEFICIENT MALE MICE.
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We have previously demonstrated that iNOS knock-out (iNOS−/−) mice have significantly increased testis weights and are partially resistant to heat-induced germ cell apoptosis. We hypothesized that iNOS may play a role in germ cell homeostasis. Groups of ten adult (10-week old) iNOS−/− and wild type (iNOS+/+) mice were studied. Germ cell apoptosis was detected by TUNEL assay. The efficiency of spermatogenesis was estimated by testicular morphology and sperm count. There was no difference in body weight between iNOS−/− (27.9±2.8g) and iNOS+/+ (28.8±3.4g) mice. In contrast, testis weight in iNOS−/− mice (TW=0.14±0.03g) was significantly increased compared to iNOS+/+ mice (0.09±0.02g). Testicular sperm count in iNOS−/− mice (9.7±1.3x106/testis) was also increased compared to iNOS+/+ males (6.9±0.3x106/testis). Morphometric analysis showed: 1) significant increase in diameter of seminiferous tubules (D) and the volumetric density of seminiferous epithelium (VSE) in iNOS−/− mice (D=219.8±7.7mm, VSE=104.1±3.7ml) when compared with iNOS+/+ males (D=200.6±6.6mm, VSE=65.4±4.2ml), 2) no significant changes in the number of Sertoli cells in iNOS−/− (13.7±2.9x106/testis) and iNOS+/+ (23.3±3.0x106/testis) mice, 3) no difference in the volumetric density of Leydig cells between iNOS−/− and iNOS+/+ mice. The incidence of spontaneous germ cell apoptosis was similar between iNOS−/− and iNOS+/+ animals. Importantly, the rate of germ cell proliferation, was significantly increased in iNOS−/− mice (PI=5.8 ± 0.7) as compared to iNOS+/+ mice (PI=4.5 ± 0.3). These results demonstrate that the deficiency of iNOS promotes germ cell proliferation in male mice. Thus, iNOS may be a physiologiogical regulator restricting the number of mature germ cells in the testes.

P5/6 – 019

IDENTIFICATION OF ANTIGENIC FOX SPERMATOZOA SURFACE PROTEINS FOR USE IN A CONTRACEPTIVE VACCINE.
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Increasing attention is being focused on fertility control as a possible technique for controlling wildlife populations, which are causing problems. Immunocastration has been suggested as a managing technique for wildlife. The aim of this work was to identify highly antigenic surface proteins on fox spermatozoa in order to use it in a contraceptive vaccine. Fox spermatozoa surface proteins were injected into 26 foxes at the beginning of the breeding season, in January, or two months before in November. Sera were sampled every month for four months. Western blot were performed on SDS-PAGE with proteins extracts from fox spermatozoa. The time course of antibody response was studied on blots. There was no significant difference in the number of recognized proteins, either between males and females or between groups injected in November or January. This number was maximal on day 90 for most of the foxes. Western blot pattern varied from one fox to another. In all, 36 protein bands were recognized between 9 and 110 kDa. By two-dimensional electrophoresis and gel-purification, we have identified and selected 6 highly antigenic proteins of molecular weight of 11.4, 14.7, 16.4, 16.4, 16.8, 16.9kDa with isoelectric points of 6.0, 6.0, 6.2, 5.5, 5.3, 5.8 respectively; and one at 97 kDa with a pI of 4.3 to 4.6. In order to achieve a better characterization of these fox antigenic proteins, their molecular sequence, their specificity and their function need to be investigated. Then they could be used in a contraceptive vaccine.
P5/6 – 020
POLYSACCHARIDES CONTAINING ARABINOSE & GALACTOSE DECREASE OXIDATIVE DAMAGE OF SPERM IN VITRO.
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Polysaccharides containing arabinose & galactose (PCAG) are abundant in plant gums. These PCAG have a membrane stabilizing effect in a variety of cell types. Studies were done to determine the effects of PCAG on bull sperm during freezing and culture. In Expt 1, ejaculates from 4 bulls at a commercial AI stud were frozen in standard egg yolk buffer (EYB) or in egg yolk buffer with PCAG (PEYB). Six straws of sperm from each bull and treatment were thawed and: 1) held at 37°C for 10 min, then evaluated for membrane lipid peroxidation (TBARS assay) and sperm chromatin damage (Sperm Chromatin Structure Assay); or 2) cultured in routine TALP medium for 24h to determine sperm survival rates. Results: Sperm frozen in EYB had more oxidative (p=0.03) and chromatin (p<0.01) damage after thawing than sperm in PEYB. Only 1 of 4 bulls had >10% motile sperm at 24h of culture for sperm frozen in EYB, whereas 3 of 4 bulls had >10% motile sperm for sperm frozen in PEYB. In Expt 2, sperm from 5 bulls (4 straws each) frozen in standard EYB were thawed, washed and placed in TALP either w/ or w/o PCAG for culture at 37°C. At 4h, sperm motility was determined and aliquots were removed to determine membrane lipid peroxidation. Results: More sperm were motile (p=0.04) and had lower oxidative damage (p=0.01) in TALP with added PCAG, than in TALP alone. Follow-up studies identified an active fraction of the PCAG between 20K and 100K which promoted sperm motility and membrane stability. Preliminary studies have shown decreased oxidative stress and chromatin damage for human sperm in culture with HTF including the PCAG. Conclusion: PCAG stabilize sperm during assisted reproduction techniques. Specifically, they appear to decrease oxidative stress and chromatin damage.

P5/6 – 021
PROSTASOMES INHIBIT THE NADPH-INDUCED SUPEROXIDE ANION PRODUCTION AND ENHANCE THE CAPACITATION OF HUMAN SPERMATOZOA.
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Prostasomes are lipid vesicles originating from the human prostate and present in seminal plasma. They have an antioxidant capacity by inhibiting NADPH-oxidase activity of blood and seminal polymorphonuclear neutrophils (Saenz et al., 2000). Their influence on the superoxide anion (O2−) production and the capacitation of human spermatozoa incubated with 2.5 μM NADPH was investigated. The study was performed on two Percoll-selected fractions of spermatozoa from normal semen. The O2− production was estimated by lucigenin-enhanced chemiluminescence, and the capacitation was assessed by the ability of spermatozoa to undergo a calcium ionophore-induced acrosome reaction (A23187-AR). The O2− production by spermatozoa of the 95% Percoll fraction and the 95-65% interface was increased by 2.5 μM NADPH (p < 0.05). This effect was partially inhibited by prostasomes (p < 0.05). The capacity of these Percoll-selected spermatozoa to undergo an A23187-AR was slightly decreased (but not significantly) by 2.5μM NADPH. Prostasomes added to this medium provoked a significant increase of the percentage of A23187-AR spermatozoa. The positive effect of prostasomes was specific to the oxidative conditions, in the presence of 2.5μM NADPH, because they inhibited the A23187-AR of spermatozoa under non-oxidative conditions (incubation without NADPH). Prostasomes have the ability to inhibit human sperm NADPH-induced O2− production, a property that could favor the sperm capacitation process under conditions of oxidative stress. SAEZ et al., 2000, Mol. Hum. Reprod., 6, (10) : 883-891.

P5/6 – 022
ADVERSE EFFECT OF SEMEN PROCESSING ON HUMAN SPERM DNA INTEGRITY IS NOT ASSOCIATED WITH INCREASED LEVELS OF SPERM FREE THIOLS.
Khaled Kamal, Keith Jarvis, Donna Phang, and Armand Zini, Toronto, Ontario, Canada.
INTRODUCTION AND OBJECTIVES: Semen is routinely processed in order to recover a highly motile sperm sub-population prior to use in ARTs. Some forms of sperm processing may adversely affect sperm DNA integrity, with unknown mechanism. We monitored the levels of sperm free thiols (SH groups) and DNA denaturation (DD) before and after density gradient centrifugation in order to explore a possible mechanism for the observed augmented sperm DD post-processing.
METHODS: Semen samples (n=43) were obtained from consecutive non-azoospermic men presenting for infertility evaluation. Samples were processed by 2-layer density gradient centrifugation. Sperm motility, sperm chromatin structure (evaluated by flow cytometry analysis of acridine orange-treated spermatozoa) and sperm SH groups were monitored before and after semen processing. Sperm chromatin integrity was expressed as the percent- age of spermatozoa demonstrating DD.
RESULTS: Following density-gradient centrifugation, mean sperm motility (±SEM) improved significantly compared to whole semen (54 ± 3 vs. 43 ± 3%, p<0.01). As previously observed, following density-gradient centrifugation, the percentage of sperm with denatured DNA increased compared to whole semen (29 ± 4 vs. 18 ± 2%, p<0.01). The level of sperm free thiold groups was lower in processed compared to whole semen (268 ± 26 vs. 534 ± 43 nmol/10⁶ spermatozoa, p<0.01).
CONCLUSIONS: Our data demonstrate that density gradient centrifugation can adversely affect sperm DNA integrity despite improved sperm motility and reduced levels of sperm SH groups in the recovered sample. These data suggest that the increased levels of sperm DD post-processing is not due to a significant degree of sperm-specific disulfides to SH group conversion.

P5/6 – 023
THE IMPACT OF OXIDATIVE STRESS ENZYMES ON SPERM MOTILITY IN PATIENTS WITH NORMAL AND ELEVATED LEVELS OF SEMINAL PLASMA LEUKOCYTES.
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The presence of elevated levels of free radicals in ejaculate is considered as one of causes of oxidative stress with the impact on sperm function. We have analyzed 114 infertile patients with normal and elevated levels of leukocytes in ejaculate and normal and/or pathologic levels of progressive sperm motility for the presence of oxidative stress enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR) as the indirect markers of free radicals presence. Standard ejaculate analyses according to the WHO criteria were done. The presence of manifest clinical infection was excluded by microbiological examination. The values of SOD, GPX and GR were examined according to the values of leukocyte number in pts with normal and pathological values of progressive sperm motility. The values of SOD, GPX and GR have statistically significant lower values in pts with normal leukocyte number and pathological sperm motility, but enzyme/Le ratio shows significantly higher values in the same conditions. These results show that SOD, GPX and GR are predominantly spent for scavenging of free radicals produced by leukocytes and, but less, defective sperms.
**P5/6 – 024**

QUALITY CONTROL OF TOTAL NON-ENZYMIC SEMINAL ANTIOXIDANT CAPACITY BY AN ENHANCED CHEMILUMINESCENCE ASSAY.


The non-enzymatic antioxidants in seminal plasma protect the sperm against oxidative insult. Accurate and reliable assessment of total non-enzymic antioxidant capacity (TAC) of seminal plasma is essential for both research and clinical purposes. The objectives of this study were to assess the inter-donor (different donor samples measured in the same day), intra-assay (running the same sample in duplicate or triplicate), inter-assay (same sample observed on different days by the same observer), and inter-observer (multiple observers on the same day with the same sample) variability using an enhanced chemiluminescence assay. Semen samples were obtained from normal donors (n=5) and from infertile men (n=5) attending the male infertility clinic. Liquefied semen samples were centrifuged at 250g for 7 minutes and aliquots of seminal plasma were stored at -80°C. TAC was measured with the luminometer set in the kinetic mode. Trolox (6-hydroxy-2, 5, 7, 8- tetramethylchroman-2-carboxylic acid), a water-soluble tocopherol analogue, was used as a standard. Results were expressed as Trolox equivalents. Significantly higher TAC [mean ± standard deviation (SD)] was seen in donors (1324 ± 191) compared to patients (542 ± 184) (P<0.0001). The intra-assay SD was 4% with an intra-assay reliability of 91% (coefficient of variation (CV)=5%). The inter-assay SD was 11% with an inter-assay reliability of 92% (CV=13%). The inter-observer SD was 115 with an inter-observer reliability of 89% (CV=13%).

Our results demonstrate that enhanced chemiluminescence assay is both accurate and reliable for measurement of TAC levels in seminal plasma.

**P5/6 – 025**

ROLE OF PYRUVATE IN OXIDATIVE-STRESS-INDUCED EFFECTS ON HUMAN SPERM ENERGY METABOLISM.

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Sperm motion is vital to fertilization. Reactive oxygen species (ROS) adversely affect sperm motility by damaging sperm membranes. Pentoxifylline (PTX) improves sperm motility via increased CAMP and reduced oxidative stress. Sodium pyruvate, an anaerobic substrate for energy metabolism in spermatozoa, increases ATP levels. How ROS affects this energy metabolism is not clear. This study evaluates the effects of sodium pyruvate, PTX, and ROS on spermatozoa. Post-thaw normal sperm were washed and aliquots incubated for various time intervals with various concentrations of sodium pyruvate, PTX, PMA-stimulated "buffy coat" leukocytes, hydrogen peroxide, or sperm wash medium (control). Sperm progression, motility viability, ATP levels, and membrane integrity (by hypotonic swelling test) were evaluated. Spermatozoa treated with ROS exhibited a dose- and time-dependent decrease in motility, forward progression and ATP levels (that was reversed by SOD, mannitol, and DMSO but not by catalase). Incubation with 5µM pyruvate and/or PTX indicated a significant increase in sperm progression from grade 1 to 3+ (P<0.001) and percent membrane integrity from 20% to 40% (P<0.003). Treatment of sperm with KCN inhibited motility and ATP levels which were reversed by pyruvate, implying that ATP generation by the citric acid cycle is independent of oxidative phosphorylation. This study also indicates that molecular targets for energy production and ROS action do not appear to be at the level of mitochondrial oxidative phosphorylation. Pyruvate protection of sperm movement suggests that oxidative phosphorylation may not be essential while other intra-mitochondrial molecular targets may be more important for oxidative stress-induced effects on sperm.

**P5/6 – 026**

ASSOCIATION OF POOR SPERM FUNCTION WITH LIPID PEROXIDATION AND PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS).

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Sperm membranes are rich in unsaturated fatty acids and they are vulnerable to lipid peroxidation (lipox) but it is unclear if lipox can explain the poor fertility associated with ROS production in sperm suspensions. We measured ROS with luminol ± 100 nM; PMA or 50 µM; NFMLP, lipox as malondialdehyde (MDA) ± a promotion with Fe²⁺/ascorbate (Fe₃Os), motility (CASA), % live sperm acrosome reacted (AR) stimulated by 1µM A23187 (A23) ± 3.6 µM pentoxifylline (P) and % morphologically normal sperm heads (CASA) in washed sperm from 73 infertility patients. The following analysis is based on NFMLP-stimulated ROS, Fe₃Os and AR after A23 + P but similar conclusions were reached from the other regimes. The relationship between ROS and lipox in sperm varied between patients and they could be divided into 3 groups: (1) No significant oxidative stress or lipid peroxidation, (ROS<25,000 BLU/s/10⁶ sperm and lipox<40nmol MDA / 10⁶ sperm), n=46. (2) Increased lipox irrespective of ROS, n=14. (3) High ROS but low lipox, n=13. Results (medians, 95% CI. (p Kruskal-Wallis)) by group were: %AR, (1) 41 (32-43), (2) 22 (14-29), (3) 24 (20-39) p<0.0001; path velocity (µm/s) (1) 37 (34-39), (2) 20 (12-30), (3) 29 (21-44) p<0.001 and % abnormal heads (1) 85 (79-89), (2) 89 (84-95), (3) 89 (84-92) p>0.46. The dichotomy in the relationship between ROS and lipox in sperm must reflect differences in anti-oxidant defences or the route of entry or activation of leukocytes in the male reproductive tract. Lipid peroxidation was strongly associated with a decreased ability to acrosome react and poor sperm motility but high ROS was associated with poor function even when lipox remained low. Head morphology was unaffected by either.

**P5/6 – 027**

LEUKOCYTE-INDUCED REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN SPERM FROM LEUKOCYTOPSPERMIC SAMPLES.

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There is much debate as to whether the origin of ROS in semen is from sperm, seminal leukocytes, or both. The objective of this study was to investigate the role of seminal leukocytes in enhancing ROS production by sperm. Semen specimens were obtained from 48 patients [leukocytospermic (n = 16), non-leukocytospermic (n = 32)] and 13 normal donors. Levels of ROS were determined in pure sperm suspensions (removal of leukocytes by Dynabeads) and after phospholipid-lysophosphatidyl ethanolamine (PEA) stimulation. Results were expressed as counted photons per minute/20 X 10⁶ sperm/mL. ROS levels (median 25% and 75%) in pure sperm suspensions were significantly higher in the leukocytospermic group (3.2 (0.65 to 6.8)) compared to the non-leukocytospermic group (0.31 (0.09 to 1.23) (P<0.002), and the donors (0.06 (0.01 to 0.23) (P<0.001). PMA induced ROS levels were significantly higher in the leukocytospermic group, 9.2 (2.7 -37.7), compared to the non-leukocytospermic group, 0.94 (0.3 -2) (P=0.0003), and donor group, 0.11 (0.03-0.1) (P<0.0002). A positive correlation was found between seminal leukocytic counts and ROS levels in pure sperm suspension (r = 0.54, P<0.0001) and after PMA stimulation (r = 0.56, P<0.0001). Our results demonstrate a strong correlation between leukocytospermia and increased potential for ROS production by sperm. In conclusion, the contribution of leukocytes to ROS production in semen appears to include not only the direct release of ROS by these cells, but also the release of ROS by leukocyte-stimulated sperm. Such stimulation may be via direct contact or mediated by soluble products released by the leukocytes.
P5/6 – 028

RELATIONSHIP BETWEEN REACTIVE OXYGEN SPECIES AND 8-HYDROXYDEOXYGUANOSINE AS AN INDICATOR OF DNA DAMAGE IN SPERMATOZOA
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Several studies have demonstrated that human spermatozoa are sensitive to Reactive Oxygen Species (ROS) induced damage due to lipid peroxidation as well as some degradation products. It was also known, that DNA of aerobic organisms was continuously damaged by reactive oxygen. One of the most common lesions is 7-hydroxy-8-oxodeoxyguanosine (8-oxodG; also called 8-hydroxydeoxyguanosine/8-OH-dG), and this oxidation product might be used as an indicator of DNA damage due to oxidation. In this study, the relationship between ROS and 8-OH-dG have been further explored in normozoospermia and asthenozoospermia semen samples. Sperm samples from 49 donors were collected and analysed by standard procedures. The measurement of ROS was carried out by chemiluminescence method utilizing luminometer, and 8-OH-dG by means of high performance liquid chromatography (HPLC). Among the 49 samples, 15 have low motility (asthenozoospermia) and 34 with normal motility (normozoospermia). Results of ROS measurement (CPM/million spermatozoa) were 24.0±14.8 in asthenozoospermia and 6.6±3.7 in normozoospermia samples, which was statistically significant (p<0.01). 8-OH-dG measurement (fmol/ng DNA) revealed a higher level in asthenozoospermia samples (8.2±1.6) compared with normozoospermia samples (3.6±2.7), which was statistically significant (p<0.05). There were positive relationship between ROS and 8-OH-dG, and negative relationship between both, ROS and 8-OHdG, with sperm motility. The results suggest that high ROS generation may induce DNA damage as shown by elevated 8-OH-dG. The damage include mitochondria DNA, and this may contribute to irreversible arrest of motility.

P5/6 – 029

ROLE OF SUPEROXIDE ANION AND HYDROGEN PEROXIDE IN ACROSOME REACTION OF BOVINE SPERMATOZOA
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Superoxide anion (O2−) and hydrogen peroxide (H2O2) are required in acrosome reaction (AR) in human spermatozoa and H2O2 induced AR in bovine spermatozoa. The aim was to study the source of O2− and the participation of O2− and H2O2 in protein kinases (PKs) and phospholipase A2 (PLA2) activation involved in the intracellular mechanisms leading to the bovine sperm AR. Capacitation was induced by 0.05μM Xanthine, 5μM/ml xanthine oxidase and 0.1mg/ml catalase (XXOC) or by 100IU/ml heparin. AR was induced by 0.1mg/ml lysophosphatidylcholine (LPC) or 0.025μM H2O2. The intracellular H2O2 concentration during LPC-induced AR in heparin-capacitated spermatozoa was determined by the p-hydroxyphenylacetic assay. AR was monitored by CTC assay. Superoxide dismutase and catalase inhibited the LPC-induced AR in heparin-capacitated spermatozoa. When heparin was used, AR was inhibited by the NAD(P)H Oxidase inhibitors 0.002μM DPI and DPEI respect to the control (p<0.05). Inhibitors of PKA, PKC and PT K (0.05μM H-89, 100nm GF109207X and 0.4μM genistein respectively) inhibited LPC-induced AR in XXOC-capacitated spermatozoa and H2O2− induced AR in heparin-capacitated spermaotoza. PLA2 inhibitor (aristolochic acid, 0.025μM) significant inhibited the AR induced by H2O2 in heparin-capacitated spermatozoa (p<0.05). In heparin-capacitated spermatozoa treated with LPC, significant levels of H2O2 were detected respect to the control (81.2±3.3 and 3.2±1.8 nM H2O2/millions cells respectively; p<0.05). These results suggest that O2− and H2O2 were required during AR in bovine spermatozoa; and H2O2 may modify the redox status. Activating PKA, PKC, PT K and PLA2 enzymes involved in the signal transduction mechanisms that lead to AR in bovine spermatozoa.

P5/6 – 030

OXIDATIVE METABOLISM AND INTRACELLULAR CALCIUM VARIA- TION IN CAPACITATED BOVINE SPERM. 
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Heparin sperm capacitation in bovine triggers a cascade of biochemical events that allow fertilization. Quercitin is an inhibitor of calcium ATPase. Isocitrate (ID1) and malate (MDH) dehydrogenases participe in sperm metabolism. Creatine kinase (CPK) is related to normal spermatogenesis and maturation. The aim was to study intracellular calcium (Ca2+), oxidative metabolic processes and CPK activity in bovine capacitation. Ca2+ was determined by FURA-2AM spectrofluorometric method. CPK, ID1 and MDH were measured spectrophotometrically by NAD(P)H variation. Sperm viability was determined by Trypan blue acrosomal integrity by differential interferential contrast microscopy and capacitation by chlorotetra- cycline. Differences were observed in Ca2+ in the presence of heparin or quercitin vs controls (p<0.05). Treatment with heparin or quercitin CPK activity decreased vs control (0.50±0.06, 0.43±0.02 and 1.47±0.45 U/10⁶ esp: p<0.05). Heparin samples decreased NADP-IDH activity vs control and quercitin treatment (1.01±0.23) x10⁻², (1.97±0.32) x10⁻² and (2.00±0.17) x10⁻² U/10⁶ esp: p<0.05). The appearance of NAD(P)H by MDH activity and IDH-NAD activity failed to show differences vs heparin or quercitin treated samples (p>0.05). The activities NADP-IDH, MDH were higher than NAD-IDH and MDH (p<0.05). The higher CPK activity of control suggests that part of the energy obtained by oxidative phosphorylation is stored in noncapacitated sperm as creatin phosphate. NADH generation during capacitation may occur through NADP-MDH and IDH isoenzymes. Raised Ca2+, oxidative metabolism NAD-linked and phosphorylation rate at substrate level would maintain an appropriate ADP/ATP ratio for motility and intracellular signals for cryopreserved bovine sperm capacitation.

P5/6 – 031

FREE THIOLS (SH GROUPS) IN HUMAN SPERMATOZOA: CORRELATION WITH SPERM DNA INTEGRITY.
Khaled Kamal*, Armand Zini, Donna Phang*. Toronto, Ontario, Canada (Presented by Dr. Kamal).

INTRODUCTION AND OBJECTIVES: Abnormal deposition of sperm protamines during spriogenesis or incomplete oxidation of sperm protamine free thiols (SH groups) during epididymal transit can lead to enhanced susceptibility to sperm DNA injury. We examined the levels of sperm SH groups and DNA denaturation (DD) in semen from fertile and infertile men. METHODS: Semen samples were obtained from consecutive non-azoospermic men presenting for infertility evaluation (n=66) and fertile men presenting for vasectomy (n=10). Standard semen parameters (sperm concentration, motility and morphology), sperm DD, sperm SH content were measured. RESULTS: Mean (±SE) levels of sperm SH and percentage of spermatozoa with DD were significantly higher in infertile compared to fertile men, respectively: SH = 1083 ± 97 vs. 570 ± 101 nmol/10⁶ spermatozoa; DD = 21 ± 7 vs. 8.9 ± 1.9%. SH and DD were positively correlated (r=0.53). As well, SH and DD correlated negatively with standard semen parameters. CONCLUSIONS: Our data demonstrate that the sperm SH content is positively correlated with sperm DD and that significantly higher levels of sperm DD and SH are found in infertile compared to fertile men. These data suggest that the enhanced susceptibility of sperm DNA to denaturation in infertile compared to fertile men may be associated with incomplete oxidation of sperm SH groups.
P5/6 – 032

BIOLOGICAL VARIABILITY OF SPERM DNA INTEGRITY IN SEMEN SAMPLES FROM INFERTILE MEN.

Khaled Kamal, Donna Phang, Keith Jarvis, Jennifer Willis and Armand Zini. Toronto, Ontario, Canada (Presented by Dr. Kamal).

INTRODUCTION & OBJECTIVES: To assess and compare the variability of conventional semen parameters and sperm DNA denaturation in repeat semen samples from infertile men. METHODS: Twenty-one (21) non-azoospermic, infertile men submitted two consecutive semen samples, two to six weeks apart. Sperm concentration, motility and chromatin structure (evaluated by flow cytometry analysis of acridine orange-treated spermatozoa and expressed as the percentage of spermatozoa with denatured DNA) were monitored. Morphology assessment was not included due to insufficient material in 20% of samples. RESULTS: Mean (± SE) sperm concentration, motility and percentage of spermatozoa with DNA denaturation were not significantly different between the first and second semen samples (12.5 ± 2.2 vs. 17.1 ± 3.3 vs 105/ml; 30.0 ± 4.1 vs. 31.0 ± 4.6% and 203 ± 2.5 vs. 19.3 ± 2.5%, respectively). Sperm DNA denaturation showed the lowest average within-subject coefficient of variation (SD/mean), followed by motility and concentration. CONCLUSIONS: Our preliminary data indicate that sperm DNA denaturation is more highly reproducible on repeat assessments than sperm concentration or motility in samples from infertile men. These data suggest that sperm DNA denaturation is a more stable biologic marker of sperm function than conventional semen parameters.

P5/6 – 033

CHROMATIN STABILITY IN SEX-SORTED SPERM.

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The integrity of DNA in sex-sorted sperm was examined using the sperm chromatin structure assay (SCSA). This assay measures resistance of sperm nuclear chromatin to low-PI-l-induced denaturation by the ratio of red to green acridine orange (AO) staining. Double-stranded, native DNA fluoresces green while single-stranded degenerate DNA fluoresces red. Sperm with damaged DNA are identified as those cells outside of the main population (COMp) expressed as % of COMp alpha (COMpAP). The objective was to determine if exposure to Hoechst 33342, 150 mW of laser illumination at 354-361 nm and/or mechanical stresses due to sorting resulted in chromatin damage as assessed by the SCSA. Samples of sperm from 2 ejaculates from 6 bulls were processed as controls (unsorted), sorted with no stain and no laser illumination, sorted with illumination but no stain and sorted with both stain and laser. Sperm were then cryopreserved by standard procedures. The mechanical stresses of sorting and/or post-sorting centrifugation only marginally increased COMpAP (3.3%) relative to unsorted, uncentrifuged control sperm (1.5%); however, neither Hoechst 33342 staining nor exposure to laser illumination during sorting increased DNA damage. Bulls differed in the susceptibility of their sperm chromatin to the mechanical stresses, but no additional damage was incurred by exposure of sperm to either dye or laser illumination.

P5/6 – 034

REDUCED SENESCENCE AND RETAINED CHROMATIN INTEGRITY IN HUMAN SPERM PREPARED BY DENSITY GRADIENT CENTRIFUGATION.

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Introduction: Anecolosal evidence suggests that human sperm retain good motility for several days at room temperature or 4°C. However, their fertilising ability and chromatin integrity are unknown. The objective was to determine whether density gradient (DG) preparation of the sperm could improve their survival and maintain their chromatin integrity over a period of days.

Methods: Aliquots of liquefied ejaculates were compared after being either extended in sperm medium or prepared on density gradients of PureSperm®. Sperm motility was assessed every 24h until it fell below 20%, and aliquots of the sperm preparations were fixed in para-formaldehyde for storage at 4°C. DNA integrity was assessed by the TUNEL assay using flow cytometry.

Results: Extended semen samples showed a high level of DNA damage on the first day (32.5%), and this increased after 24 h storage. In contrast, DG sperm showed 13% TUNEL positivity on the first day, rising to 16% after 24 hours storage. In five of the nine DG preparations, TUNEL positivity remained ≤20% for a further 48 hours, while motility remained >20% for up to 6 days.

Conclusion: DG preparation increased the mean survival time of motile sperm from 24 h to 72 h. Nuclear DNA damage was lower in DG prepared sperm and did not rise significantly during storage.

P5/6 – 035

RIBONUCLEIC ACID CONTENT IN SPERMATOZOA: MOTILE VERSUS NONMOTILE.

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Introduction: Previous studies demonstrated the presence of ribonucleic acid (RNA) in mature, ejaculated sperm. Abnormal sperm have significantly more RNA than normal sperm. However, this study did not take into account differences within the same ejaculate, rather between samples. Objective: Our purpose was to determine whether there are any differences in total RNA content between motile and nonmotile populations within the same ejaculate. Design: Comparison of RNA content between motile and nonmotile sperm. Methods: Sperm samples were separated by density centrifugation into motile and nonmotile populations. Total RNA was extracted from sperm via the QiaGen (Valencia, CA, USA) mini RNA kit. Total RNA (micrograms of RNA per million sperm cells) recovered per sperm population from each ejaculate was determined by spectrophotometer and calculated as follows: (Absorbance at 260nm) X (Conversion factor) X (Dilution factor). The conversion factor for RNA is 0.04 micrograms/microliter per optical density unit at 260nm. Total RNA purity was calculated as the absorbance at 260nm divided by the absorbance at 280nm. Data were analyzed by Students t test. Results: A total of 33 ejaculates from different men underwent RNA extraction and analysis as described. All samples had absorbance readings between 0.1 and 1.0 at 260nm and were determined to be accurate. Total RNA purity estimates for all samples were within the acceptable ratio of 1.7 and 1.9. Nonmotile sperm population (0.49 +/- 0.07 micrograms/million cells) was significantly higher (P<0.1) than the amount found in the motile sperm population (0.10 +/- 0.02 micrograms/million cells). Conclusion: Ribonucleic acid content is significantly altered in non-motile (abnormal) sperm, and this alteration may be the result of some defect at the transcriptional/translational level.
P5/6 – 036
IMPORTANCE OF SEMEN BANKING IN PATIENTS WITH SYSTEMIC DISEASES.
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Chronic non-testicular illnesses and immunosuppressive/ cytotoxic therapy for non-malignant diseases can permanently suppress spermatogenesis. The problem is on the rise among young men receiving treatment for systemic diseases with therapies that can alter their fertility status. The objective of this study was to determine the usefulness of cryopreservation in a group of patients with non-malignant, non-testicular conditions who may require immunosuppressive or cytotoxic therapy. Their pre-treatment semen quality was assessed and compared to the pre-freeze and post-thaw semen parameters of a group of healthy donors. Semen specimens were obtained from 25 donors and 23 patients with varying clinical diagnoses [autoimmune disorders (n = 11), kidney diseases (n = 4), diabetes (n = 3), and others (n = 5)]. All specimens were cryopreserved by a standard freezing procedure. Pre-freeze and post-thaw sperm motion characteristics were measured. Patients and donors matched in age and ejaculate volume. Patients' pre-freeze and post-thaw sperm count and motility, though significantly lower than donors (p = 0.001), matched the WHO reference range for semen parameters. Semen quality (pre-freeze and post-thaw) was similar among patient groups divided by their diagnoses. Thirty percent of patients (7/23) had greater than 40 million motile sperm after freezing (mean ± SD 120.6 ± 155, range 41 – 448 X 10^6). These patients can pursue a simple intrauterine insemination procedure for assisted reproduction. The remaining patients had adequate motile sperm count for in vitro fertilization or intracytoplasmic sperm injection methods (6.0 ± 0.38, 0.5 – 23.3 X 10^6). We recommend semen cryopreservation in these patients prior to their treatment to enable future pregnancies.

P5/6 – 037
ENHANCED SPERM-MEDIATED GENE TRANSFER BY ELECTROPORATION: EFFICIENCY, RELIABILITY AND EXPRESSION OF TRANSGENE
Plasmids containing a salmon insulin-like growth factor gene (pMTIGF) constructs were used to test the efficiency of gene transfer using salmon sperm cells as a vector. Electroporated salmon sperm cells were more efficient and more reliable than untreated sperm in picking up exogenous DNA and subsequently transferring the DNA into salmon embryos. Evidence suggest that some of the exogenous DNA was the taken up DNA retained its integrity as demonstrated by the polymerase chain reaction (PCR). The transferred exogenous DNA was detected by PCR over a period of 16 months. The percentage of fish containing the transgene randomly sampled over a period of 16 months was over 30%. Integration of the transgene into the host genome was not detected by Southern blot analysis, but expression of the transgene was detected by reverse transcription polymerase chain reaction (RT-PCR) in the blood of two fish. This suggests that messenger RNA for the insulin-like growth factor gene was produced in the blood cells.

P5/6 – 038
ZYMOGRAPHIC EVALUATION OF PROACROSIN/ACROSIN SYSTEM IN SPERMATOZOA OF TWO MARSUPIAL SPECIES, THE BRUHSHELL POSSUM (TRICHOSIRUS VULPECULA) AND THE TAMMAR WALLABY (MACROPS EUGENII).
A zymographic procedure using gelatin-sodium dodecylsulphate polyacrylamide gel electrophoresis (gelatin-SDS PAGE) has been optimized to study proacrosin/acrosin system in two species of marsupial, the brushtail possum (Trichosurus Vulpecula) and tammar wallaby (Macropus eugeni). Acid extracts of both the ejaculated and the epididymal sperm were used to estimate the esterolytic activity using spectrophotometer and the proteolytic activity using gelatin-SDS PAGE. An acid extract of brushtail possum epididymal sperm show only one band of protease digestion on gelatin-SDS PAGE which has an approximate molecular weight of 50 kDa while tammar wallaby epididymal and ejaculated sperm extract showed four protease digestion bands of approximate molecular weights 45, 44, 36 28 kDa. Preincubating the gels with 50 µM benzamidane completely inhibited the protease digestion, indicating that these are trypsin-like proteases. The study of activation profile of acid extract in both these species using spectrophotometric assay as well as gelatin-SDS PAGE procedure demonstrated the presence of zymogen form of acrosin called proacrosin. Acrosin activation to acrosin occurs maximally within 30 min and at pH 8.0. The total acrosin activity of sperm present in both these species was found to be several-folds the activity found in eutharian sperm. No differences were found either in proacrosin activation profile or total acrosin activity isolated from either epididymal or ejaculated spermatozoa in both these species. The importance of the study in relation to fertilization process in these species is discussed.

P5/6 – 039
DIRECT EFFECTS OF MAST CELL PRODUCTS ON HUMAN SPERM MOTILITY.
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Introduction: Mast cells are involved in fibrotic disorders of different tissues. The number of testicular mast cells is increased in cases of spermatogetic arrest. Treatment of infertile men with mast cell blockers was shown to increase semen quality. The purpose of the present study was to examine in vitro-effects of two mast cell products on human sperm motility. Material and methods: Ejaculates from fertile men (n=15) were washed twice with human tubular fluid medium. Spermatozoa were resuspended in medium and incubated with different concentrations of trypsinase (10/100/1000 ng/ml) or chymase (1/100/1000 mg/ml). After 60, 180 and 180 minutes, motility was determined by computer assisted semen analysis. In addition, sperm motility was measured after two washing steps for elimination of tryptase/chymase.
Results: Motile spermatozoa were significantly (p<0.01) reduced after 10 min of incubation with 1000 ng/ml trypsinase. After 60 min, motility was significantly (p<0.001) reduced in samples incubated with 100 and 1000 ng/ml tryptase. A significant (p<0.05) decrease of sperm motility was also observed after incubation with 1000 ng/ml chymase for 60 min. Incubation for 180 min had no further effects. Elimination of tryptase increased sperm motility significantly (p<0.05).
Discussion: Tryptase is present in human seminal plasma and was shown to affect spermatozoa in vitro. Therefore, mast cell products may influence sperm quality indirectly by impairment of spermatogenesis and directly by effects on spermatozoa.
**P5/6 - 040**

**LOCALISATION OF PROLACTIN RECEPTORS IN THE REPRODUCTIVE TRACT OF THE RAM.**

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Prolactin is thought to be involved in the regulation of testicular function. High prolactin concentrations during the non-breeding season of rams are thought to maintain basal testicular activity and allow a rapid response to rising gonadotrophin levels at the onset of the breeding season. Prolactin receptors (PRL-R) have previously been localised to germ cells and Leydig cells of testis from sexually mature rams. In this study we have investigated the localisation of PRL-R in the testes of rams throughout sexual development. PRL-R localisation in the epididymis was also determined. Sections of Bouins fixed tissue underwent high temperature antigen retrieval prior to immuno localization of PRL-R using a polyclonal antibody directed to the extracelullar domain of rat PRL-R. Immunoreactive peptide was visualised with hydrogen peroxide and diaminobenzidine. In testicular tissue PRL-R were localised to pachyctene spermatocytes and all stages of spermatids. This germ cell staining was not seen in testicular sections from rams in the early stages of sexual development. No staining was visualised in Leydig cells. In the epididymis, PRL-R were localised exclusively within the luminal edge of epithelial cells. Localisation of PRL-R to pachyctene spermatocytes as well as round and elongating spermatids suggests prolactin may be acting as a regulator of cell division or differentiation within the testis. In addition, PRL-R localisation in the epididymis implies the presence of prolactin in tubular fluid. These results support a role for prolactin in the regulation of testicular function and provide evidence for an additional role within the epididymis.

**P5/6 - 041**

**INFLUENCE OF TESTICULAR SEROTONERGIC, MELATONINERGIC, AND CATECHOLAMINERGIC SYSTEMS ON TESTOSTERONE AND CAMP PRODUCTION.**

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The aim of this work is to establish heterologous interactions between the testicular serotoninergic, melatoninergic and catecholaminergic systems and its influence on testosterone (T) and cAMP production from purified Golden hamster Leydig cells. hCG-stimulated (100 mIU/ml) T production was evaluated (29.4 ± 1.3 ng/10⁶ Leydig cell), with or without serotonin (5-HT), epinephrine (E), norepinephrine (NE) and agnuside(aga)/agonist(ant) of these receptors. An inhibitory action of 5-HT and 5-HTIA/2A ags were described. In contrast, E/NE and α1/Bs stimulate T production. Ketanserine (ket). 2AaNT, reverts the effect of 5-HT (5-HT: 21.9 ± 1.5: 5-HT+ket: 26.9 ± 1.0); ag2- (DOI: 21.8 ± 3; DOI+ket: 30.2 ± 4), Bag (isoproterenol (iso) 36.4 ± 0.8; iso: 29.4 ± 2.4) and α1- ag (phenylephrine,(phen): 37.0 ± 0.3; phen+ket:31.9 ± 1). Nevertheless, ket. failed to revert the stimulatory action of α1/B adrenergic agonist on hCG-stimulated cAMP production (hCG:31.8 ± 1); ket: 38.2 ± 2, iso: 47.6 ± 0.4; phen: 49.4 ± 0.7; ket+iso:41.3 ± 5 ket+phen: 43.1 ± 0.8) (pmol cAMP/10⁶ Leydig cells, * p<0.05). However, pMppi, 1Aant, reverses the effect of SHT (5-HT+pMppi: 25.5 ± 0.5) and the 1Aag. (DPAT: 22.5 ± 5; DPAT+pMppi: 29.8 ± 0.7) on T and cAMP production, without alterations in the response to α1/Btag († * p<0.05). In summary, our studies demonstrate that α1/B, melatonin and α1/B adrenoceptors acts in the tests as local regulators of cAMP and T production, and b) cross-competition between their receptor systems.

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**P5/6 - 042**

**HORMONAL REGULATION OF MONOMERIC AND DIMERIC INHIBIN PRODUCTION IN HUMAN SEMINIFEROUS TUBES.**


Inhibin B (Inh B) is a glycoprotein produced by Sertoli cells under the regulation of FSH and gonadotroph factors. In boys, both monomeric (Pro-aC) and dimeric Inh B circulate in high concentrations after birth. The aim of this study was to assess the regulation of inhibin production in newborn testis. Testicular tissue was obtained from a 1-month-old patient with partial androgen insensitivity. Tissue samples were frozen in liquid nitrogen before being used in in vitro assays. Interstitial cells (IC) and seminiferous tubules (ST) were derived from birth. ST were cultured for 6 days with medium changes every 48h. The following stimuli were added to the media: FSH (100ng/ml), E2 (100ng/ml), insulin (100ng/ml), and inhibin α (10ng/ml). DEX (100uM) was added to the media. Inhibin B production in newborn testis (basal 41.6±1.9 vs 15.1±0.7ng/ml; p<0.001). Only ICMM was able to stimulate Inh B production (70±1.4ng/ml; p<0.001). Although basal Inh A levels were below the detection limit of the assay, Inh B levels were stimulated by ICMM to 53±6.2 and 79±4.8pg/ml, respectively. These results show that in newborn testis: Pro-aC and Inh B are produced in high amounts, independently of FSH stimulation; Inh A is also produced and it is stimulated by cytokines; unknown factors produced by IC are involved in the stimulation of Inh B production.

**P5/6 - 043**

**CRYPTORCHIDISM: SEASONAL VARIATIONS IN GREECE.**

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Objective: To examine seasonal trends of cryptorchidism in Greek population. Methods: Data on five hundred eighty three males with true isolated cryptorchidism born between 1995-1998 were received and analysed from three major paediatric centre databases. All 208,912 live-born boys born during the same period of time were used as a comparison group. Seasonality by month of birth was evaluated using both Edwards' model with adjusted frequencies and exact q, and Walter-Elwood's method with exact q. Results: Both tests resulted in comparable and consistent findings. The application of Edwards' test showed a maximum incidence rate in late March and a minimum in late September (c2=19.34 at 2 df; p<0.005). The application of Walter-Elwood's test also showed a maximum incidence rate in late March and a minimum in late September (c2=16.24 at 2 df; p<0.005). Conclusions: Since no significant differences in daylight length are found among seasons in Greece, the detection of a statistically significant variation may suggest that factors other than light are involved in the pathogenesis of cryptorchidism. Seasonal alterations in the frequency of a certain infectious disease impairing placental function and thus disturbing sexual development of the fetus may be given as a possible example of an alternative mechanism contributing to the seasonal variations of cryptorchidism. This point of view is further supported by the similarity of our results to those reported by other European countries of different longitude and geographical width.
P5/6 - 044
SERUM INHIBIN B CONCENTRATION AS A DIAGNOSTIC AND PROGNOSTIC MARKER IN IDIOPATHIC OLIGOZOOSPERMIA.

Sertoli cells display activities which are vital for sperm production. Of those, secretion of some hormones, growth factors and transporting proteins appear to be uniquely regulated by Sertoli cells and may be considered as good markers of their activity. In this study, Sertoli cell function was assessed under basal and hp-FSH (225 i.u.) plus hCG (1500 i.u.) stimulated conditions in normozoospermic (n=11), oligo- with normal FSH (n=32) and oligo- men with raised FSH (n=12). Sampling was carried out at 0, 3, 6, 24 and 48 hours with determinations of serum FSH, LH, inhibin α and B, transferrin, IGF-I, IGF-1BP3, T and E2. Furthermore evaluation of sperm response to rec-FSH (150 i.u./48 hours x 6 months) in oligo- men was made in relation to basal or stimulated inb-B in order to assess its predictive value. The results obtained can be summarized as follow: 1. A significant rise of inhibin B, IGF-I, T and E2 was noted in normozoospermic men at 24 and 48 hours. In oligo- groups the 24/48 hours response did not differ from basal values whereas the area under the response curve was lower than in normo- men. 2. In oligo- with normal FSH a correlation between functional sperm fraction (FSF) vs FSH concentration (r: 0.462, P<0.004) and inb-B vs total testicular volume (r: 0.369, P<0.05) was noted, but when all cases were grouped together some significant correlations were found (FSH vs inb-B r:-0.513 P<0.0011; FSH vs inb-B 48 r:-0.558 P<0.0001; FSF vs inb-B 48-0 r:-0.375 P<0.002; inb-B vs FSF r: 0.448 P<0.0003; inb-B:FSH vs FSF r: 0.417 P<0.001). Furthermore, the ratio of inb-B:FSH discriminated better than FSH or inb B alone between the groups. 3. A pilot trial of rec-FSH administration in 17 oligo- men showed a doubling of basal FSH at 6 months.

P5/6 - 045
MORPHOMETRIC ANALYSIS OF THE TESTIS IN ADULT MULES.
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The mule (Equus mulus mulus, 63 chromosomes) is a sterile domestic animal that results from the breeding of donkey (Equus asinus, 62 chromosomes) vs mare (Equus caballus, 64 chromosomes). Because the testis determining factor is supposedly located in the short arm of the Y chromosome, the mule represents a potential recipient model for transplants of spermatogonial stem cells derived from the donkey. In the present study, testes from seven young adult mules were fixed with 4% glutaraldehyde. The testis tissue was embedded in plastic and routinely prepared for histological and histometrical analysis. Seminiferous tubule (ST) and Leydig cell (LC) volume density (%) in mules were respectively 63% and 6%. The mean ST diameter was 131μm, while 47 μm of ST were found per gram of testis. The individual LC volume and the LC nucleus volume were 1800μm³ and 250μm³, respectively. LC and Sertoli cells looked apparently normal and their number per gram of testis were approximately 30 millions and 116 millions, respectively. Analysis from several hundreds of ST cross-sections per animal showed that 72% of ST presented evident lumen while in 5% of them no lumen was observed. Concerning the evolution of spermatogenesis the ST containing up to primary spermatocytes, spermatagonia and Sertoli cell-only represented 77%, 18% and 5%, respectively. No more advanced germ cell type than spermatocytes was found. Apoptotic cells were observed in 55% of the ST examined, being located mostly in the region of the seminiferous epithelium occupied by spermatocytes. To our knowledge, this is the first study to perform a more detailed and comprehensive functional analysis of the tests in mules.

P5/6 - 046
PURIFICATION OF RAT LEYDIG CELLS: INCREASED YIELDS AFTER UNIT GRAVITY SEDIMENTATION OF COLLAGENASE DISPERSED INTERSTITIAL CELLS.

Procedures for purification of Leydig cells have facilitated studies of their regulatory biology. A multistep procedure, that includes a filtration with nylon mesh (100 micron pore size) to separate interstitial cells from the seminiferous tubules, combining centrifugal elutriation and Percoll density gradient sedimentation, has been used to obtain a 95% enrichment of rat Leydig cells. However, the number of recovered Leydig cells by this procedure represents only a small fraction of the 25 million that exist on average in the adult rat testis. The objective of this study was to test whether the yield of purified Leydig cells might be enhanced by substitution of unit gravity sedimentation (S method) for the filter step (F method). The number of purified Leydig cells was 1.9-fold higher for the S method than for the F method, with no differences in purity assessed by β3-HSD histochemical staining. Leydig cell clusters were also found in greater numbers with the S method both after collagenase dispersion and at the end of the purification. No differences were seen in T production or in the number of macrophages present in the Leydig cells that were prepared by the two methods. These results indicate that the new method recovers greater numbers of Leydig cells by collecting clustered Leydig cells that are systematically eliminated when a filtration step is used. Supported in part by NIH HD32588 and the Population Council.

P5/6 - 047
TESTOSTERONE PRESERVES MITOCHONDRIAL RESPIRATION OF IN VITRO CULTURED RAT LEYDIG CELLS EXPOSED TO LIPOPOLYSACCHARIDE.
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Introduction. To study the mechanism(s) by which inflammation damages testicular function, the effects of bacterial lipopolysaccharide (LPS) on in vitro cultured rat Leydig cells (LCs) were studied. Material and methods. Purified LCs of adult rats were incubated short-term with medium alone, LH (10 μU/ml) and Testosterone (T, 30 nM) in the presence or absence of LPS (1-100 ng/ml). After 3 h, media were collected for assay of T (by RIA) and nitric oxide (NO, by Griess reaction1), and mitochondrial respiration was tested (MR, by evaluating the conversion of MTT to formasan). Results. Administration of LPS: 1) did not influence LCs T secretion; 2) reduced spontaneous NO secretion by 30% and increased the inhibitory effect of LH and exogenous T on NO secretion2; 3) reduced MR to <10%. T, either exogenously added or endogenously secreted (by LH stimulation), partially preserved MR. Conclusion. Short-term administered LPS does not affect T secretion, but impairs NO secretion and, more markedly, MR. T itself, by preventing these detrimental effects, seems to exert a protective role against testicular inflammation. 1 Valenti S et al, Int J Androl 1999, 22:336; 2 Valenti S et al J Endocrinol Invest 1999, 22 (S4):70.
P5/6 - 048
EXTRACELLULAR MATRIX PROTEINS MODULATE TESTOSTERONE PRODUCTION BY LEYDIG CELLS IN VITRO. E.S. Diaz*, E. Pellizzari*, S. Meroni*, S. Cigorraga*, L. Lustig1, B. Deduchus1. 1Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires. 2Centro de Investigaciones Endocrino-metabolicas, CONICET. Buenos Aires, Argentina.

The aim of this work was to study the role of extracellular matrix (ECM) proteins on hCG-stimulated testosterone (T) production in Leydig cells (LC). LC isolated from adult rat testes were cultured on plates uncoated or coated with collagen IV (Col-IV), fibronectin (Fn) or laminin 1 (Ln 1) (1-8 µg/well) in the presence of hCG (10 ng/ml) during 3h. Morphological changes in LC cultured on ECM proteins were observed as well as a significant increase of cell adhesion in these cultures. A decrease in T production was observed when LC were cultured on plates coated with Col-IV or Fn (4-8 µg/well) as compared to uncoated plates. T (ng/10⁶ cells), Col-IV 55±5.7*or Fn: 80±17 * vs uncoated plates 91.0±1.5 (X±SE, *P<0.001). Results of MTS viability test excluded a cytotoxic effect of ECM proteins. No significant differences in the specific ³²P hCG binding among LC cultured on plates uncoated or coated with ECM proteins were seen. In addition, a decrease on extracellular cAMP production (fmol/10⁶ cells) was observed in LC cultured on Col-IV (225±7.5*) or Fn (304±3.9*) when compared to uncoated plates (480±2.5) (X±SE, *P<0.001). Laminin 1 did not modify LC testosterone and cAMP production. Results suggest that collagen IV or fibronectin used as cell substrates inhibit hCG-stimulated Leydig cell testosterone production. This effect might be exerted through the regulation of cAMP levels.

P5/6 - 049

The aim of this work was to study the involvement of the Fas-Fas L system in germ cell death and detachment from the seminiferous epithelium in rats undergoing an autoimmune orchitis (EOA). EAO was induced by active immunization with testis homogenate and adjuvants. Rats injected with saline and adjuvants were used as controls (C). Animals were killed at 35, 50, 80 and 120 days after the first immunization. The testicular damage, focal at 50 days and severe after 80 days, was characterized by a scarce interstitial mononuclear cell infiltrate and by germ cell sloughing (mainly spermatids and spermatocytes). The number of germ cells in the lumen of epididymal tubules of rats with EAO, increased significantly (after day 80) with time and the development of the testicular damage, in contrast with C rats presenting a normal testicular histopathology. Quantitative immunohistochemical results revealed an increased expression of Fas (after day 50) and Fas L in spermatocytes and spermatids in rats with EAO, compared to rats from C group. TUNEL method performed on paraffin sections showed a 2.4 and 24-fold increase in the number of apoptotic germ cells in rats with EAO at 50 and 80 days respectively, compared to C group, at each time. The overexpression of Fas and the increase in the number of apoptotic germ cells in rats with EAO preceded the detachment of germ cells from the seminiferous epithelium. Results suggest that the Fas-Fas L system is involved in the apoptosis of germ cells of rats undergoing an autoimmune orchitis.

P5/6 - 050
POSTNATAL CHANGES IN LACTATE DEHYDROGENASE OF BUFFALO TESTIS
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Lactate dehydrogenase (LDH) was histochemically localized and its specific activity as well as the pattern of its isoenzymes in the testis of 1-72 months and above old buffaloes were related to specific ages and to the germ cell maturation. With age, the histochemical enzyme activity increased in the tubular cells. Specific activity of LDH was maximum in 1 month stage decreased significantly and became constant after twelve months followed by a further increase after 48 months. Decrease in the total LDH activity after 1 month was correlated with the spermatogonial and spermatocyte differentiation as well as with the decrease in the density of HM1 and HM2 bands. Appearance of X' band (specific in buffalo testis) in 12 month old animals was correlated with the significant increase in the number of pachytene spermatocyte which prevented the further decrease in the total LDH activity after 12 months. However, the increase in the number and density of LDH-X bands from 36 months onwards was correlated with the activation of spermatogenesis and with the increase in the total LDH activity after 48 months.

P5/6 - 051
CHANGES IN PROTEINS AND ENZYMES DURING DEVELOPMENT AND MATURATION OF BUFFALO TESTIS
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In view of the lack of the systematic work on the development and maturation of testis in buffalo- an important dairy animal of India, the present correlative morphological (cell types) and biochemical study has been made from 1-to-48 -and 72- months age groups .The biochemical estimations include total proteins (soluble, membrane bound ), alkaline phosphatase (soluble-SAKP, membrane bound-MAKP) and 17β- hydroxysteroid dehydrogenase (17β- HSDH). Active spermatogenesis occurs at the age of 36 - 48 months. Soluble protein content is maximum at 1 and 3 months (imperural period) of age but decreases gradually, thereafter, while membrane bound proteins do not change significantly. Higher activity of soluble alkaline phosphatase is noted from 36 (prepubertal period) to 48 months (pubertal period) while activity of membrane bound alkaline phosphatase was highest from 1 to 9 months (imperural period) and at 48 months of age; however, activity of 17β - HSDH significantly increases from 30 to 48 months. It is concluded that proteins may be used for differentiation of testicular cells during impuberal period; MAKP may be responsible for increased metabolic processes during impuberal period; high activity of MAKP, SAKP and 17β - HSDH from prepubertal to pubertal period may be related to increased testosterone production.
P5/6 – 052
THE EARLY CHANGES OF SEMINIFEROUS TUBULAR BOUNDARY ZONE & ELASTIC FIBERS IN VASECTOMIZED YOUNG RATS : LIGHT & ELECTRON MICROSCOPIC STUDY
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Vasectomy is a contraceptive method widely used in the world, after which seminiferous tubules degeneration and interruption of spermatogenesis take place. The boundary zone and elastic fibers play an important role in the function of the seminiferous tubules, therefore we investigated these structures in young Wistar Albino rats after bilateral vasectomy. The animals were set in 4 groups for the experiment:
1) Sham group (only abdominal incision, n=20)
2) 2 weeks after vasectomy (n=20)
3) 6 weeks after vasectomy (n=20)
4) 12 weeks after vasectomy (n=20)
The animals were sacrificed and testes biopsies were taken for light and electron microscopic investigations. Hematoxylin-Eosin and Resorcin-Fuchsin staining methods were used for light microscopic comparisons. Sperm granulomas were observed macroscopically in all groups. Microscopically significant and increasing seminiferous tubules degenerations were seen whereas in the same chronology a decrease of elastic fibers (and sometimes loss) was noticed parallely to changes of the boundary zone (thickening of basement membrane, myoid cell granulations etc.). The absence of elastic fibers in the wall of seminiferous tubules are known to be a sign of prepuberual atrophy. The loss or absence of the fibers in our investigation is based on the same decrease of active spermatogenesis in vasectomized rats which may lead to a generalized atrophy of the testes in these animals.

P5/6 – 053
KING’S ‘CRISS-CROSS’SIGN IDENTIFIES MALIGNANT TUMOURS OF THE TESTIS
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INTRODUCTION: Developments in ultrasound probe technology have lead to improvements in both B-mode and colour Doppler imaging using high frequencies. This has followed more detailed evaluation of intratesticular lesions and a more confident assessment of benign and malignant disease. We have evaluated a series of testicular lesions paying particular attention to lesion vascularity, to ascertain the nature of abnormality. MATERIAL AND METHODS: Over a 16-month period a total of 1351 consecutive patients underwent scrotal ultrasound for the usual indications. A new high frequency probe (1518w, acuson sequoia) was used and the testicular ultrasound and doppler imaging was performed. The Ultrasound diagnosis of the abnormality was correlated with histology. RESULTS: A total of 30 focal testicular abnormalities were identified. There were 21 tumours, 2 arterio-venous malformations, 4 testicular infarcts (focal and diffuse) and 3 epididymal abscesses. The high frequency probe clearly identified all these lesions and demonstrated disorders ‘Criss-Cross vascularity(King’s Criss-cross sign) in 17 testicular cancers(seminoma and teratoma)(81%). Four tumours(19%) which did not exhibit this pattern had the following histological diagnosis: secondary(adenocarcinoma) from prostate cancer, Leydig cell tumour, benign teratoma, and secondary from myeloid leukemia. For the diagnosis of common testicular tumours(seminoma and teratoma) the vascular sign had a sensitivity and specificity of 100%. CONCLUSION: Although small in number, this distinct vascular sign allows confident diagnosis of seminoma and teratoma. There are however, no specific differentiating features between these two tumour types.

P5/6 – 054
C-KIT DEFICIENCY NOT ONLY AFFECTS SPERM PRODUCTION BUT ALSO ALTERS SPERM FERTILISING ABILITY.
D Royere, F Cuért, V Laurent Cadoret, JJ Panchier, MT Hochereau de Reviers Research Unit "Reproductive and Behavioural Physiology" INRA / CNRS / University Reproductive Biology Unit. Dept of Ob-Gyn & Human Reproduction, University Hospital, Tours, France.
Introduction C-kit / Stem cell factor have been reported to play a crucial role both in developing gonad and onset of spermatogenesis. Using a c-kit transgenic haplodeficient mice model, we have confirmed the alterations of spermatogenesis leading to a drastic decrease of all germ cells except type A spermatagonia. Our aim was to evaluate the fertility of these animals both in vivo and in vitro, as compared to inbred wild males. Materials and methods Testis, seminal vesicles, epididymis weight were evaluated at 3 different ages (45 days, 3 & 12 months) for 8 animals of both genotypes. Total sperm count from the epididymis, as well as percent vitality were measured in the same animals. Mating involved heterozygous males with wild females, as well as females and males of wild genotypes, or heterozygous females with wild males. In vitro fertilisation involved 10 to 12 wild females, with their pooled oocytes and 2 males of each genotype for each of 4 experiments. Statistics were done using variance analysis followed by mean comparison or contingency table. Results & conclusion Comparing both genotypes, we observed a 50% decrease in testis weight of heterozygous males (3 months: 55.4 + 19.2 vs. 108.5 + 11.6 mg, p<0.001) with a deeper decrease in total sperm count (13.7 + 2.1 vs. 5.5 + 2.3 millions, p<0.001). Neither total body, nor vesicle seminal or epididymal weights differed between both genotypes. In vivo fertility tests showed a drastic decrease in pregnant female rate for heterozygous males as compared to wild males (6.5% vs. 15 %, p<0.010).

P5/6 – 055
FERTILITY AFTER CHEMOTHERAPY (CT) AND RADIOTHERAPY (RT) FOR TESTICULAR GERM CELL TUMORS
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OBJECTIVE: To evaluate the long-term impact of different CT regimens and RT on fertility for testicular germ cell tumors. MATERIAL AND METHODS: From 1983 to 1996, 134 orchietomized pts. with testicular cancer, of whom 82 (61%) underwent CT and 52 (39%) intradermaphagic RT, were selected by the requirement of pre- and post treatment sperm count (SC). In all pts. SC were assessed >1 year after treatment, with the median follow-up time of 38 months. SC were classified as normo- (NS - >10x10⁶/ml), oligo- (OS 1 to 9x10⁶/ml) and asospermic (AS - <1x10⁶/ml). The CT regimens used were: PVB-28 pts; BEP-23 pts; EP-18 and CEB-13 pts. Fifty three pts. received 4 cycles of CT and 29 pts. >4 cycles. The prognostic importance of age, CT regimen, number of CT cycles, pretreatment SC, disease stage, radiation dose and serum HCG level on the recovery of SC were analyzed using uni- and multivariate techniques. RESULTS: Of 134 pts. 70 (52%) were NS and 34 (25%) OS before CT and RT. This rate was similar in both groups. Of 70 NS pts. 42 (60%) recovered to NS, 16 (23%) to OS and 12 (17%) remained NS after 1 year of treatment. Recovery rates to NS and OS were the same for CT and RT groups (p=0.05). Overall, 44% of the pts. recovered to NS and the chance of recovery increased with the time since the treatment. NS was achieved in 25% of pts. after 2 years compared with 53% recovery rate after 6 years (p<0.05). In both uni- and multivariate analysis pretreatment SC, number of CT cycles and RT dosage significantly affected recovery rates to OS and NS. CONCLUSION: Our data suggest that fertility following germ cell CT and RT is the same for both modes of treatment and recovery of spermatogenesis depends on pretreatment SC and dose administered.
P5/6 – 056
SEASONALITY OF BIRTH IN PATIENTS WITH NEOPLASIAS OF THE TESTIS AND THE PROSTATE.
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Seasonality of birth has been observed in the incidence of some genetic disorders such as Down syndrome. Some of the most important xenobiotic metabolizing enzymes, N-acetyltransferases, are also involved in the metabolic pathway of pineal indolamines. These molecules are responsible for regulating many aspects of circadian and circannual cycles. They also exert important effects on reproduction, and on lactogenic hormones. The latter action being important for the immune function. Seasonality among births in Indiana has a slight peak in July/August and a trough in April. This reflects what is observed for the USA population in general. Cancer and control patient data was collected for admissions into University Hospital for October 1998-April 1999. Among patients admitted to University Hospital, seasonality of birth was observed to include peaks in March, August and October with troughs in February, April and November. In men diagnosed with testicular cancer, the peaks were in March (P=0.001), October (P=0.024) and December (P=0.001), with troughs February (P=0.04) and June (P=0.001). In men diagnosed with prostate cancer, the peaks were in May (P=0.025) and September (P=0.001), with troughs March (P=0.013) and October (P=0.001). For both types of neoplasias, statistical analysis reveals significant differences in the patterns of seasonality of birth as compared to the pattern observed for all male patients admitted during the same period of time. These preliminary results appear to indicate that seasonal changes in the hormonal milieu might imprint the fetus' immune system. This could then modulate the ability of an individual to combat specific types of neoplasias.

P5/6 – 057
FERTILITY AFTER TESTIS CANCER
Objective: to evaluate the fertility after testis cancer treatment (surgery, radiotherapy, chemotherapy) over the last twenty years.
Patients & Method: From 1979 to 1998, 314 men with testicular cancer and having given sperm samples (for conservation) before radiotherapy or chemotherapy treatment, have been investigated. Reproductive events have been collected throughout a mail questionnaire and telephone interviews; histology stage of testis cancer and complete description of treatments have been obtained throughout several medical files. The participation rate was over 90%. Results: Among the 277 men for whom we were able to obtain valid informations, 138 have tried to have a child after testis cancer treatment, with a success for 91 (66%). In univariate and multivariate analysis, the significant risk factors for not having a pregnancy were a history of cryptorchidism, a sperm count before testis cancer below 10 M per ml, and having had a surgery with a retroperitoneal lymph node dissection. No relation was found between fertility and having had a radiotherapy or a chemotherapy for testis cancer. By using the time to obtain a pregnancy (in months) as an indicator of reproduction ability, we found that for couples having decided to obtain a child, 60% conceived within one year; a result which is less than those observed in overall population (90%) but nevertheless relatively optimistic. Conclusion: In our study performed in a large and exhaustive French regional serie, the fertility of patients with testis cancer was not substantially modified by the radiotherapy or chemotherapy treatments.

P5/6 – 058
HIGHER SPERM CONCENTRATION IN SOUTHERN SWEDEN AS COMPARED TO DENMARK CORRESPONDS TO A LOWER RISK OF TESTICULAR CANCER
J Richthoff, J Malm and A Giwercman, Department of Urology, Clinical Chemistry, Malmo University Hospital.
It has been suggested that sperm concentration has declined during the past few decades. During the same period of time testicular cancer (Tc) and possibly congenital abnormalities, of male genital have become more common. A common cause of these amounts has been suggested, and focus has been directed toward environmentally derived compounds, with hormone-like action affecting male gonad in early foetal life. Five times higher risk of Tc in Denmark as compared to Finland is associated with a significantly higher sperm concentration among the Finnish males. This supports the hypothesis of correlation between Tc and sperm production. However, the discrepancy between the Danish and Finnish males may not only be due to environmental and lifestyle related, but also genetic factors. We therefore compared semen parameters in geographically and genetically closely related populations in Southern Sweden and in Denmark. We asked 300-army conscripts form Southern Sweden, aged 18 years, to provide a semen sample. Same selections and methodology were appealed as in a recently published Danish study (Anderson et al Human Reproduction vol. 15 no 2 pp 366-372,2000). We have found 30 % higher sperm concentration and 42% higher total sperm count in Swedish males as compared to the Danish. Both age and seasonal distribution as well as the time of abstinence were comparable. There was no difference in sperm motility. In a population with 3 times lower risk of Tc as compared to Denmark, we found 42% higher sperm counts. These results point to environmental or lifestyle related factors as possible determinants of sperm production and Tc, since a genetical discrepancy between these two populations seems to be unlikely.

P5/6 – 059
CELLULAR MANIFESTATIONS OF UNDESCENDED TESTES DEPEND UPON THE ETIOLOGY OF CRYPTORCHIDISM BUT NOT THE ABDOMINAL LOCATION PER SE
Testicular carcinoma in situ (CIS), which precedes germ cell tumors, has been associated with existence or a history of testicular maldescent in 20% to 30% of human cases. The objective of this study was to determine if the abdominal location of the testis by itself causes transformation of germ cells and why only some undescended testes are affected. Five groups (n=4-6) of pregnant Dutch-Delted rabbits were treated on alternate days between gestation days 15 and 30 with anti-androgenic chemicals – p.p'. DDT, p,p'-DDE (100 mg/kg body wt. oral), or flutamide (50 mg/kg; s.c.); estrogenic chemical ocytphelin (150 mg/kg; oral); or nothing. Groups (n=4-6) of neonatal male pups from untreated litters were either implanted with estradiol (0.5 mg; 60-d-release), actively immunized against GnRH, surgically rendered cryptorchid, or left untreated (control). Of the pups exposed in utero, 2 of the 7 DDT, 4 of the 12 DDE, 2 of the 9 flutamide and 1 of the 4 ocytphelin pups were unilaterally cryptorchid. All animals from the 3 groups treated neonatally were bilaterally cryptorchid. Light and electron microspopic evaluation at 24-26 wk revealed presence of atypical germ cells in the undesended testes of DDT-, DDE-, ocytphelin-, and estradiol-exposed animals but not in others. These atypical cells exhibited morphological hallmarks of CIS, which included large nuclei with irregular contours, meandering nucleoli, swollen mitochondria, unusual cytoplasmic inclusions and occasional mitotic figures. Thus CIS could result from various actions of chemicals, which might include endocrine disruption, but not from abdominal location of the testis per se.
P5/6 – 060

SERTOLI CELL TUMOR ASSOCIATED WITH KLINFEFFERIS SYNDROME.

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The association between Klinefelter’s syndrome (KS) and neoplasia is rare. Incidence of breast carcinoma in KS is increased to similar levels to the general female population. Occasionally, germinomas have been described. We communicate the association between KS and Sertoli cell tumor that, to our knowledge, has not been reported previously. A 15-year-old boy with KS (karyotype XXY+) presented a hard enlargement of the left testis (8 ml). The testis had been cryptorchid until 9.8 years, and descended to the scrotum after treatment with human Corionic Gonadotropin (hCG). Three of his grandparents have died of breast or lung cancer. β-HCG and α fetoprotein were negative and the ultrasound revealed a heterogeneous mass of 1.27 x 1.23 cm in the lower testicular pole. On radical orchidectomy, a solid whitish tumor of 1.3 cm was found, entirely surrounded by testicular parenchyma. Histologically, it showed spindle shaped cells arranged in clusters, and a small area of focal tubular pattern with bland cytologic features and low mitotic activity. The capsule was ill-defined and signs of vascular or capsule infiltration were absent. The tumor was considered as a Sertoli cell tumor. Immunohistochemical findings supported the diagnosis. Anti-Müllerian hormone (AMH), a specific marker of Sertoli cells, and Inhibin β usually expressed in Sertoli cell tumors were mildly positive. Epithelial, germinal, Leydig cell or myogenic origin were discarded by negative immunodetection of Epithelial membrane antigen, Placental alkaline phosphatase, Smooth muscle actin and 3β hydroxysteroid dehydrogenase, respectively.

P5/6 – 061

ANEUPLOIDY IN SPERMATOZOA AFTER PEB CHEMOTHERAPY USED IN TESTICULAR CANCERS

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Objective: to evaluate the chromosomal consequences of the PEB chemotherapy (Cisplatin, Etoposide and Bleomycin) on sperm cells during the period of recovery of spermatogenesis in patients treated for testicular cancer. Patients & Method: we studied sperm cells aneuploidy in five testicular cancer patients, 6 to 18 months after the PEB chemotherapy, using fluorescent in situ hybridisation with chromosomes 7, 16, 18, X and Y specific DNA probes. Semen specimens from five healthy fertile sperm volunteer donors were used as controls. Results & discussion: We observed a significant increase frequency of diploidy and disomy for the chromosomes 16, 18 and XY in treated patients compared to a healthy control group. A recent study revealed an increased aneuploidy in sperm cells in one patient during the course and one year after PEB delivery. The same results were not observed later, 2 to 13 years after treatment. The PEB chemotherapy used in testicular cancers has improved long-term survival of these patients. Following an azospermic period, sperm cells recovery is generally observed after treatment and a child conception project is possible. The consequences of aneuploid spermatozoa could be spontaneous abortion, stillbirth or birth defects children. Patients and practitioners must be informed about these facts to discuss the interest and the duration of contraception after PEB treatment. Conclusion: Our study demonstrates a significant increase in sperm aneuploidy in PEB treated patients, in the 18 first months after chemotherapy. Disappearance with time of this PEB side effect must be confirmed. These data must be used in genetic counselling for testicular cancer treatment.

P5/6 – 062

DYSGENETIC GONADS MAINTAIN PREINVASIVE GERM CELL CARCINOMA PREDOMINANTLY AT 46, XY KARYOTYPE


We have investigated if numerical and structural aberrations of sex chromosomes (NSAS) predispone to gonadal dysgenesis (GD), testicular carcinoma in situ (CIS), gonadoblastoma (GDA) or germ cell carcinoma (CA). 40 subsequent patients with GD, aged 3 mo. - 19 yrs, were studied. 27 of them were 46,XY (67.5%), one - 46,XX (2.5%), 11 had NSAS (27.5%) and one was 46,XY with autosomal translocation (7,9) (2.5%). Female internal, ambiguous or female external genitalia were present in all cases. Gonads were located abdominally or in the inguinal canal. Bilateral gonadectomy was performed. The presence of placental-like alkaline phosphatase (PLAP), a marker of CA and CIS was identified immunohistochemically in germ cells.

Pure GD (bilaterally streak gonads) was 46,XY in 69.2% cases, while 30.8% had NSAS. Mixed GD (unilateral testis) was 46,XY in 76.9% cases, while 23.1% had NSAS. Partial GD (bilateral testes) was 46,XY in 54.5% cases, 27.3% had NSAS, one had 46,XX and one - 46,XY (7,9).

The incidence of neoplastic lesions are presented in the table:

<table>
<thead>
<tr>
<th>Karyotypes</th>
<th>CA: n (%)</th>
<th>CIS: n (%)</th>
<th>GDA: n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,XX</td>
<td>(23)</td>
<td>(3/7)</td>
<td>15 (55.5)</td>
</tr>
<tr>
<td>NSAS</td>
<td>(11)</td>
<td>0 (0.0)</td>
<td>4 (36.4)</td>
</tr>
</tbody>
</table>

n - number of cases, CA - dysgerminoma

Tests in 46,XX and 46,XY (7,9) revealed the presence of CIS as well.

The results show that although NSAS and 46,XY (7,9) predispose to GD and neoplastic lesions of dysgenetic gonads, the highest incidence of both phenonmena is associated with normal male karyotype. Therefore it seems that abnormal organogenesis of the testes has no uniform ethiology and may per se cause the development of CIS, GDA and gonadal CA in intersexual individuals.

P5/6 – 063

RELATIONSHIP OF INSULIN, SEX HORMONES, LEPTIN AND C21 STERIODS IN MEN WITH REPRODUCTIVE DISORDERS.

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The investigation was designed to determine the relationship between leptin level and age, BMI, insulin, sex hormones and C21 steroids in men with different reproductive dysfunction (hyper- and hypogonadotropic hypogonadism, varicoceles). We studied 69 patients (age range 18-57, mean 33±1 yr.) In these subjects BMI ranged 20-40 kg/m2, total testicular volume 0.4-4.2 cm3, insulin 2.7-117 ml/L, testosterone (T) 1.9-36.8 nmol/L, estradiol 49-489 pmol/L, LH 0.6-41 IU/L, FSH 0.5-33.5 IU/L, prolactin 75-2500 ml/L, cortisol 114-1389 nmol/L, corticosterone 5.7-72.6 nmol/L, 17-OH-progesterone 0.2-7.6 nmol/L, DHEA 8-61.3 nmol/L, DHEAS 0.9-7.8 umol/L and leptin 0.8-106 ng/ml. Statistical correlation between studied parameters was computed by means of the Spearman rank-correlation, which does not require normal distribution. Insulin significantly correlates with BMI (R=0.48, p=0.0003), leptin (R=0.27, p=0.02), and correlates negatively with T (p=0.0007). Significant negative correlation was observed between leptin level and T (p=0.0044), and positive correlation with DHEA (p=0.007), DHEAS (p=0.01), corticosterone (p=0.045), and BMI (p=0.021). Higher leptin levels were registered in patients in who androgen deficiency was present along with obesity (18.6 nmol/L). In patients with high insulin level and obesity the leptin level increased to a minor extent (10.6±2.1 nmol/L). Our data suggest that leptin-testosterone linkage in males is stronger than leptin-insulin. The latter relationship has complex character with participation of other hormones and/or metabolic factors, possibly blood glucose level.
Abstracts – Poster Session 5/6

P5/6 – 064

EXPRESSION OF LUTEINIZING HORMONE (LH) SUBUNIT GENES IN THE RAT EPIDIDYMIS AND SEMINAL VESICLE
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INTRODUCTIONS: Recent studies revealed the novel expression of luteinizing hormone (LH) in the rat testis and ovary, though the biological role of the gonadal LH is not clearly understood yet. The present study was performed to explore whether LH gene is expressed in other reproductive organs of male rat. MATERIALS AND METHODS: Adult male rats (Sprague Dawley strain) were sacrificed, then the reproductive organs including testis, epididymis and seminal vesicle were collected. The organs were applied in both RNA extraction and soluble protein preparation. Presence of the transcripts for LH subunits was demonstrated by reverse transcription-polymerase chain reaction (RT-PCR) and southern blot analysis. To assess the LH production in these organs, LH immunooassay (RIA) was employed. RESULTS: The cDNA fragments for LH beta subunit corresponding to the exons found in rat pituitary and testicular LH were amplified from the rat epididymal and seminal vesicle samples. Similarly, the mRNA for the common alpha-subunit in the accessory sex organs was confirmed by RT-PCR. In LH RIA, significant amount of immunoreactive LH molecules (irLH) was detected from crude extracts of rat epididymis and seminal vesicle. The competition curves with increasing amount of tissue extracts were parallel with those of pituitary and standard LH reference, indicating the irLH is similar to authentic rat pituitary LH. CONCLUSIONS: Our data demonstrate that genes for LH subunits are expressed in the rat accessory sex organs. These findings suggest that the local LH might act not only as an autocrine and/or paracrine factor in the regulation of epididymal and seminal vesicle function but also as an exocrine factor in male reproduction.

P5/6 – 065

SODIUM VALPROATE MONOTHERAPY AND SEX HORMONES IN MEN
Shasha Jain*; Neelam Jain; Department of Neurosurgery S.M.S. Hospital Jaipur, Indian Redcross Hospital, Jaipur, India
Antiepileptic drugs affect various endoconal functions including sex hormone levels. In this study, the effect of sodium valproate monotherapy on sex hormones has been evaluated in sexually asymptomatic patients of primary generalised tonic and clonic seizures. Twenty five young male patients with seizures and twenty five controls were subjects in the study. Sodium Valproate monotherapy (20-30 mg. perkg.) was given in the patients to control the seizures. None of the patients had recurrence of seizures during one year of follow up. Serum levels of sodium valproate, total serum testosterone, leutinizing hormone, follicle stimulating hormones were estimated initially, before starting the valproate therapy. These were repeated at three month intervals for one year. Patients on sodium Valproate therapy showed minor changes in serum sex hormone level. The total testosterone level fell significantly at the end of one year, however LH, FSH, and serum prolactin levels remained unalterded. Thus it was concluded that sodium valproate has a potential for decreasing the levels of testosterone after prolonged administration.

P5/6 – 067

DISTURBANCES OF DAILY PITUITARY-GONADAL AND -ADRENAL AXIS HORMONES SECRETION IN MALES WITH LIVER CIRRHOSIS (LC) AND CHRONIC ACTIVE HEPATITIS B (CAH):

D Rajmaniuk*, B Marek*, Z Ostrowska*, B Kos-Kuda*, and K Wrzus-Korczala*, Departments of Pathophysiology & Endocrinology, Clinical Biochemistry, Physiology, Silesian Medical Academy, Zabrze, Poland
The liver plays a significant role in maintenance of homeostasis of the organism by way of endocrine system. The aim of the study was to examine circadian secretion rhythm of testosterone (T), estradiol (E2), sex hormone binding globulin (SHBG), folitropine (FSII), lutropine (LH), corticotropin (ACTH), cortisol (F), androstendione (A-4) and dehydroepiandrosterone sulfate (DHEAS) in patients with chronic liver function lesions. Studies were performed in 30 patients with CAH and LC. Blood for studies was collected every 3 hours. Both in LC and CAH groups significant increase diurnal E2, T, and SHBG secretion were observed. In patients with CAH diplacement of daily rhythm of ACTH in later hours was observed however, in group with LC total loss of daily rhythm and essential decrease of daily secretion of this hormone in relation to control (C) as well to CAH groups was noted. Loss of daily periodicity as well as distinct decrease of total daily F secretion in CAH and LC groups in relation to C group was observed. In groups with CAH and LC significant decrease of daily DHEAS secretion in relation to C group was noted. Daily secretion of A-4 was significantly increased in LC group in relation to C group as well as in CAH. Conclusions: 1. Essential circadian changes of hormones concentrations of pituitary-adrenal and -gonadal axis in blood and disturbance in proper rhythm of their secretion are present in patients with liver function lesions. 2. Hepatic lesion, especially in case of LC leads to significant increase of androstendione concentration in blood, which can be one of the essential reasons for hyperestroneism observed in this disease.
Abstracts – Poster Session 5/6

P5/6 – 068
HORMONAL REGULATION OF FSH POLYMORPHISM IN ANORCHID BOYS.

Follicle-stimulating hormone is produced and secreted in multiple molecular forms with different biological activities. In the male, GnRH and androgens modulate this heterogeneity. The aim of this study was to determine the effect of GnRH and testosterone administration on serum FSH polymorphism in anorchid boys. Eight prepubertal (chronological age (c.a.) range: 0.8-13.4 yr; Group I) and five pubertal (c.a.range: 13.5-19.2 yr; Group II) patients were studied and compared to 15 age-matched normal boys. Five prepupal patients were studied after i.v. administration of GnRH (100 μg); five pubertal patients were studied over a period of 7-14 days after the administration of i.m. exogenous testosterone. Serum FSH isoforms were isolated by preparative isoelectric focusing and lectin chromatography (Concanavalin-A). In Group I, basal FSH isoforms were found in a pH range 2.5-5.5 and post GnRH in 2.0-5.5 whereas in controls the corresponding values were: 3.0-5.0 and 3.5-5.5 respectively. In Group II, under basal conditions: 2.5-5.5 and in controls: 3.0-4.5; post testosterone pH range was: 2.5-5.5. Con-A chromatography isolated FSH isoforms bearing complex (Lb and WB) and high mannos/hybrid type oligosaccharides (FB). The predominant FSH isoforms recovered in Group I, under basal conditions were Lb (30%) and FB (22%); in controls: WB (44%) and FB (38%). In patients, there was no change after GnRH administration whereas in controls, Lb FSH isoforms increased from 17% up to 41%. In Group II, despite the high variability observed among patients, testosterone was able to restore the physiological profile. In conclusion, GnRH is unable to release less syallated FSH isoforms in anorchid boys; however, testosterone is able to increase the proportion of FSH bearing highly branched oligosaccharides.

P5/6 – 070
THE POSSIBLE ROLE OF PROLACTIN IN REGULATION OF ADRENAL ANDROGEN FUNCTION
MV Karyakin *, AS Abloyan *, and NP Goncharov, National Center for Human Reproduction, National Research Center for Endocrinology, Moscow, Russia.

It was shown in some studies that in patients with hyperprolactinemia elevated levels of adrenal androgens, for example dehydroepiandrosterone sulfate (DHEA)-S were observed. The aim of the present study was to investigate the possible role of prolactin (Prl) in adrenal steroidogenesis. 445 men from 17 to 55 years (mean age 32.4± 6.8years) with different stage of varicocele participated in the study. Basal blood samples were assayed for Prl, progesterone (P), 17α-hydroxyprogesterone (17-0HP), cortisol (F), DHEA and DHEA-S. In all patients normal levels of Prl for this age group were found. The medium level of Prl was 233.1±89.0 munit/l, P - 2.2±1.5 mmol/l, F - 465.2±171.6 mmol/l, DHEA - 27.2±12.0 nmol/l, DHEA-S 3600.8±1362.4 nmol/LPrl levels correlated positively with P (r=0.16, p=0.001), F (r=0.19, p=0.000), DHEA (r=0.15, p=0.001), and DHEA-S (r=0.20, p=0.000). Our data suggested that in men of reproductive age Prl could be involved in control of DHEA-S and other adrenal steroids synthesis.

P5/6 – 069
SIGNIFICANT REGIONAL DIFFERENCES IN SEXUAL HORMONE CONCENTRATION IN BLOOD SAMPLES OF MILITARY CONSCRIPTS
B.Zilaitiene1, N.Jorgensen2, A.G.Andersen2, N.E.Shalobieebko2, V.Matusievicius1, R.Zalinekienis1; 1Institute of Endocrinology, Kaunas University of Medicine, Kaunas, Lithuania; 2Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark.

The aim of the study was to determine regional differences in semen quality 18-26 years old men (military conscripts) in Denmark and Lithuania. Blood samples were drawn to measure sexual hormones level. Methods. Blood samples were obtained in 444 Danish and 208 Lithuanian military conscripts. All hormone determinations were done in the laboratory of Rigshospitalet, Copenhagen. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were determined by time resolved-immunofluorometric assay (DELFIA, Wallac, Finland). Inhibin B was determined using a specific two-sided enzyme immuno- metric assay. Results. The main results of hormone investigation are as following:

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Denmark</th>
<th>Lithuania</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosteron (nmol/l)</td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
<td></td>
</tr>
<tr>
<td>Inhibit B (pg/ml)</td>
<td>196.8±68.2</td>
<td>233.1±89.7</td>
<td>0.0000000</td>
</tr>
<tr>
<td>LH (μU/ml)</td>
<td>4.07±1.75</td>
<td>4.17±1.54</td>
<td>n.s.</td>
</tr>
<tr>
<td>FSH (μU/ml)</td>
<td>3.73±2.64</td>
<td>3.60±2.51</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>30.85±17.47</td>
<td>39.61±17.35</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>94.48±31.70</td>
<td>91.21±30.43</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

A significant difference in the concentration of testosterone, inhibit B and SHBG was observed in men from two different populations. Calculated free testosterone concentration was higher in Lithuanian men too. These results seem to be important to support the hypothesis about the regional differences in main parameters of male reproductive health. Conclusion. We detected a significant difference in inhibit B, testosterone, SHBG and free testosterone concentration.

P5/6 – 071
EFFECTS OF LEUPROLIDE TREATMENT ON PLASMA FSH LEVELS AND TESTIS DEVELOPMENT
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Leuprolide is a very potent GnRH agonist utilized to suppress gonadotropins. The aim of the present study was to investigate the effects of one single neonatal (day 0) s.c. injection (0.25mg) of leuprolide acetate (TAP Pharmaceuticals Inc.) on FSH levels and testis development. Treated and matched control Wistar rats were sacrificed at 1d, 2d, 3d, 5d, 10d, 14d, 20d, 25d, 30d, 43d, and 100d after injection and had their testes fixed with 4% glutaraldehyde. Before sacrifice, blood samples were taken to measure FSH levels using RIA. Testes were embedded in plastic and routinely prepared for histomorphometric analysis. From day 1 to day 30, FSH levels were lower in treated rats compared with controls, the differences significant (p<0.05) at 3d, 20d, 25d, and 30d. The gonadosomatic index (GSI) in treated rats was significantly higher (p<0.05) at 1d, suggesting that FSH levels were increased shortly after injection. For the duration of the study, significant correlations were observed between GSI and FSH levels (r = 0.71), and between tubular diameter and FSI (r = 0.66). Decreased Sertoli cell proliferation in treated animals most likely resulted in a decreased tubular diameter (p<0.05) during the periods that germ cells were actively proliferating (30d, 43d, and 100d). The testis size and Sertoli cell number in adult treated rats were decreased by approximately 25% and 20% (p<0.05), respectively. Leuprolide suppressed FSI levels for about one month. Financial support: FAPEMIG, CNPq and NIH HD 35494.
P5/6 – 072
INCREASED PROCOLLAGEN α1 (III) EXPRESSION IS AN EARLY INDICATOR OF FIBROSIS IN THE AGING PENIS.
Mahadevan Rajasekaran, Armen Kayan*, Manoj Monga, UCSD Medical Center, San Diego, CA.
Altered penile trabecular connective tissue results in corporal veno-occlusive dysfunction and age-related erectile dysfunction. Elevated procollagen α1 (III) (PIIIP) is an early indicator of fibrosis in the liver and lung. We evaluated age-related changes in cavernosal smooth muscle in the Brown-Norway rat, and correlated these changes with the expression of fibrogenic markers PIIIP and TGF-β1. Young (4 mo) and old (21 mo) Brown-Norway male rats (NIA, Bethesda, MD) were euthanized and penile tissue rapidly harvested for histological and molecular analyses. Formalin-fixed tissues were sectioned and paraffin sections used for Masson’s Trichrome staining. Total RNA was extracted using TRIZOL reagent, specific primers (TGF-β1, PIIIP) were custom synthesized and an Access RT-PCR System (Promega) was used for mRNA expression analysis. PCR reaction products were analyzed on a 1.2% agarose mini gel system and a computerized image analysis system was employed to quantify the band intensity. Masson’s Trichrome staining of the penile mid shaft tissue revealed no difference in cavernosal smooth muscle content between the age groups. Penile tissues of both young and old animals showed the gene expression for both TGF-β1 and PIIIP. No age-related difference was observed in TGF-β1 mRNA levels, whereas PIIIP expression showed a 2-fold increase in the penile tissue of old animals. Elevated PIIIP expression may be an early marker of tissue fibrosis in the aging penis. PIIIP may contribute to the abnormal age-related structural alterations in the erectile tissue. Early detection of fibrotic changes could facilitate timely therapeutic intervention before irreversible tissue damage occurs. Supported in part by an AFUD/AUA summer scholarship to AK.

P5/6 – 073
HORMONE REPLACEMENT THERAPY FOR PADAM: OUTCOME AND SAFETY (MIDDLE EAST MULTICENTER STUDY)
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Objectives: To evaluate the efficacy of testosterone replacement therapy for the treatment of PADAM symptoms in men 50-70 years old. Design and methods: Open, non-comparative, multicenter, project of observation & monitoring performed in 7 centers in 6 Middle Eastern countries. TT, SHBG, FSH, LH, PRL, PSA were measured & repeated 2 months after HRT. A rating score was used for male climacteric symptoms. Eighty-seven patients were included in this study on the basis of an abnormal PADAM rating score associated with a low TT, FT or Free androgen index. Results: Mean PADAM scores showed a significant improvement (p<0.05, Wilcoxon Matched pairs test) on the 2-month follow up visit. Eighty-two percent of patients showed improved PADAM scores. No significant changes in serum levels of PSA, PRL, FSH, were detected (Paired t-test). No significant side effects were noted. Conclusions: HRT appears to be beneficial & safe in the treatment of PADAM symptoms, during our early follow up period.

P5/6 – 074
MAST CELL IN THE AGING HUMAN TESTIS.
Rodriguez H, Insula A, Diaz G, Ossandon E., Laboratory of Histoembriology, Morphology Program. ICBM. U de Chile.
In man, the presence of mast cells has been associated with infertility and spermatogenic defects. In the healthy testis, there is a scarce number of interstitial and peritubular mastocytes. They are believed to appear after primary testicular lesions. Their presence and the activation of metalloproteins are signs of local lesion, derived from pathological process or aging. The presence and distribution of mastocytes in human senile testicular interstitium in the presence of normal seminiferous tubules, was analyzed. Nine patients aged between 74 and 86 years, all of whom have descendant, were orchietomized. Tests were fixed in bouin’s fluid for 12 hs and processed for routine histology, and stained with toluidine blue. Mastocytes were counted in the interstitium and in perivascular or peritubular locations. Histological evolution of seminiferous tubules in the same area was done. In all cases there was abundance of mastocytes preferentially located in the interstitium and associated to seminiferous tubules with histopathological changes such as tubular blockage, vacuolization, epithelial damage or decreased epithelial height (hypo spermatogenesis).

P5/6 – 075
INCREASE IN MISCARRIAGE RISK WITH MATERNAL AND PATERNAL AGE: RESULTS OF A MULTICENTER EUROPEAN STUDY.
PF Thonneau, E de La Rochebrochard. Research Group in Human Fertility, La Grave Hospital, Toulouse, France.
Background The risk of spontaneous abortion has been shown to increase with maternal age. It is unknown whether paternal age has a confounding effect on this association. Methods We evaluated the risk of miscarriage with maternal and paternal age by analysing 3,174 last pregnancy outcomes reported in a European study conducted from 1991 to 1993. Ages were defined using a unique variable, couple age, in a logistic model stratified for country, number of the pregnancy, time to pregnancy, female smoking, male smoking, history of miscarriage, history of ectopic pregnancy and history of induced abortion. Findings If the man was under the age of 40, the risk of miscarriage was higher if the woman was over 35 years of age. If the man was over the age of 40, the risk of miscarriage was higher if the woman was over 30 years of age and was much higher if the woman was over 35 years of age. Other risk factors were a long time to pregnancy, a history of miscarriage and a history of ectopic pregnancy. Conclusions The risk of miscarriage increases with maternal and paternal age. The effects of the two partners’ ages interact and reinforce each other.
**P5/6 – 076**

**EFFECT OF AGING AND HORMONAL REPLACEMENT THERAPY ON RAT TESTES: LINKS WITH APOPTOSIS, MITOCHONDRIAL FUNCTION AND OXIDATIVE STRESS.**

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Ageing of testes is often accompanied by a loss of germ cells via apoptosis and endocrine dysfunction. This study was designed to investigate the mechanisms underlying age-related decline in spermatogenesis. Rats of 6, 12, 16, 26 month old were divided into 4 groups (Cp) according to onset of hormonal and morphological dysfunction. Group II was subdivided into a, band c. Subcutaneous testosterone (T) and oral vitamin E were given for Cp III a, b respectively. Plasma, bioactive T, LH and FSH were measured. The tests were weighed and prepared for immunohistochemical detection of DNA fragmentation and confirmation of the results by electron microscopy. Homogenized tests were used for measuring telomerase mRNA oxidized to reduced glutathione ratio. Cytochrome C oxidase in mitochondrial fraction was estimated. 8-hydroxy-deoxyguanosine (8-OHdG) is a biomarker for DNA damage by oxidative stress was estimated by HPLC. The results showed that at the beginning of 16 month old rat, testicular weight, bioactive T and LH decreased whereas FSH and the proportion of regressed tests increased with ageing. FSH declined at 24 month old. Apoptotic index was significantly higher in group III, IV. The striking morphological changes in mitochondria of Sertoli & Leydig cells started in group III suggested possible intrinsic limitation that prevent germ cell from renewing themselves and died by apoptosis. The concomitant significant decrease of mitochondrial cytochrome C oxidase and significant increase in 8-OHdG in group IIIC&IV support the mitochondrial role in age-related germ cell loss via apoptosis. The slight improvement of the these parameters in groups IIIa&b indicate that endocrine ageing in males is inevitable.

**P5/6 – 077**

**AN EQUATION FOR THE CALCULATION OF BIOAVAILABLE TESTOSTERONE.**

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The physiologically important fraction of serum testosterone may be that which is not bound to SHBG, but includes the fraction loosely bound to albumin. We have measured bioavailable testosterone (BT) by radioimmunoassay after ammonium sulphate precipitation of SHBG in 131 serum samples from males aged 60-88 years. From these data we have calculated the dissociation constant (KD) of the testosterone-SHBG complex, which has a mean value of 10.3 x 10^-3 M. Using this value we have derived an equation which permits the calculation of BT (cBT) from the measurement of the total concentration of testosterone and SHBG. cBT = a [CT - CS - (10.3 x 10^-3)] + 2 x (10.3 x 10^-3)CS. CT = total concentration of SHBG and CT = total concentration of testosterone. BT and cBT values were strongly related (R=0.82, y-int 0.46 nmol/L), but the calculation consistently provided higher values than the assay (P<0.001).

Mean concentrations of TT; BT and cBT were 16.5 ± 5.8 (range 4.0 - 33.6), 3.7 ± 1.1 (1.0 - 7.4) and 4.6 ± 1.5 (1.3-8.6) nmol/L, respectively. In male Red Cross blood donors (19 - 65 years) BT and cBT were inversely related to age (R=-0.56, P<0.01) but not to SHBG after controlling for the effect of age. "Normal ranges" for calculated and measured BT were comparable across all ages. In a cohort of men (n=84), BT (R=-0.23, P=0.05) and cBT (R=-0.21, P<0.05) but not TT or free testosterone index (100/SHBG), were inversely related to muscle strength and muscular fatigue on grip-strength dynamometry, a relationship that was independent of age. From a clinical standpoint the BT assay is difficult to automate, as opposed to TT.

**P5/6 – 078**

**SERUM TESTOSTERONE LEVELS IN PROVEN FERTILE, NORMOZOOSPERMIC MEN: A BETTER GROUP FOR "NORMAL" VALUES?**

MS Bornman, M Roux, W Vermaak, S Reif. Departments of Urology and Institute of Pathology, University of Pretoria, Pretoria, South Africa.

Low serum testosterone levels must support the supposed clinical manifestations of androgen deficiency before the physician may consider androgen substitution. Reliable references values for serum testosterone (s-T) are, therefore, crucial. In this study hormonal analyses were performed in 129 men (aged 20 to 49 years) with sperm counts >20 mill/million (WHO 1992) on the day of vasectomy. The men were subdivided into three groups - 20-29 years (n=30), 30-39 years (n=83) and 40-49 years (n=16). The mean values of total serum testosterone (s-T), the free androgen index (FAI) and sex binding globulin (SHBG) of all three groups were in the lower range of normal for our laboratory. The values were statistically significantly lower than the control values for the laboratory (collected from medical students and staff) (test for one mean). In spite clear evidence of normal exocrine function of the testes (semen quality), measurements of endocrine function seemed lower. This emphasises the importance of using the correct reference group for normal values.

**P5/6 – 079**

**SERUM LEVELS OF ADRENAL AND TESTICULAR STEROIDAL HORMONES IN AGED MALES**

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It has been reported that serum levels of adrenal androgens and gonadal androgens of the male decrease gradually with age after their thirties. Of late decades, life span of males as well as females increased and duration of the senescence became long in many males. In this study, we evaluated change of serum levels of adrenal and gonadal steroids with age in elder males. Blood samples were obtained from 80 male patients aged 60 or older than 60 at 10:00 to 12:00 in the morning. Their chief complaints were lower urinary tract symptoms. Of the patients, 5 patients who were treated with hormones were excluded. Serum concentrations of total and free testosterone(T), dihydrotestosterone(DHT), dehydroepiandrosterone sulfate(DHEAS), and cortisol were measured by RIA. Change of each hormone with age was evaluated statistically. Age of the elder males in this study ranged 60 to 90 and the mean age was 71.1 year old. Serum concentrations (Means±SD) of total T, free T, DHT, DHEAS, cortisol were 389±144 ng/dL, 7.6±3.0 pg/mL, 0.87±0.46 ng/mL, 1063±689 ng/mL, 13.5±3.6 micro-g/dL, respectively. Serum levels of free T and DHEAS decreased with age significantly (free T: p=0.026, DHEAS: p=0.044). Serum levels of total T, DHT, Cortisol did not change with age (total T: p=0.21, DHT: p=0.61, Cortisol: p=0.93). These data suggest that production of androgens but not glucocorticoids in the adrenal might decrease to a large extent during senescence and that production of androgens in the testis might decrease to a small extent and other mechanisms decrease serum level of free T during the period.
P5/6 – 080

REJUVENATION OF AGED LEYDIG CELLS IS POSSIBLE IN A RODENT SPECIES S.M.L. Chamindrani Mendis-Handagamaa,1, In-shik Kimb and H.B. Siril Ariyaratnet. Departments of Comparative Medicine and Animal Science, College of Veterinary Medicine, The University Of Tennessee, Knoxville, TN 37996 U.S.A.

We investigated the possibility of rejuvenating aged Leydig cells (LC) in Brown Norway rats. 7 groups of rats (n=8 per group), i.e. 3, 6 and 12 month old rats and four groups of 19 month old rats were used. 19 month old rats were implanted subdermally with Alzet mini osmotic pumps containing saline (control), luteinizing hormone (LH, 24ug/day), thyroxine (THY, 5ug/day) and LH+THY (24ug/day+5ug/day, separate pumps), respectively, at 18 months age (for four weeks). Morphometric parameters and steroidogenic function of LCs in these rats were examined using routine techniques. Testis volume was unchanged among all treatment groups. The absolute volume of LC per testis was highest at 3 and 6 months, lowest at 19 months (saline) and intermediate in LH-, THY- and LH+THY-treated groups. The number of LC per testis was not significantly different (P>0.05) among all treatment groups. The average volume of a LC was highest at 3 and 6 months and lowest at 19 months (saline). These values in LH- and THY- treated rats were significantly higher than those of 19 month rats (saline), but were lower than those of 12 months and LH+THY-treated; no difference (P>0.05) was seen between the latter two groups. LH-stimulated testosterone secretory capacity per testis and per LC in vitro was first reduced (P<0.05) at 12 months and a further reduction occurred at 19 months (saline), values of LH- and THY-treated 19 month old rats were similar to those of 12 months and values for LH+THY-treated rats were similar to those of 3 and 6 months. These findings demonstrated that the treatment of aged Brown Norway rats (12 months) with either LH or THY was able to restore the testicular steroidogenic potential to those of 12 months of age, and treatment of LH+THY was able to restore this potential to those of 3 and 6 months of age. (supported-UT Minkel and COE grants)

P5/6 – 081

REJUVENATION OF AGED LEYDIG CELLS IS POSSIBLE IN A RODENT SPECIES S.M.L. Chamindrani Mendis-Handagamaa,1, In-shik Kimb and H.B. Siril Ariyaratnet. Departments of Comparative Medicine and Animal Science, College of Veterinary Medicine, The University Of Tennessee, Knoxville, TN 37996 U.S.A.

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P5/6 – 082

POWER TRAINING AND TESTOSTERONE SUPPLEMENTATION IN OLDER ANDROGEN DEFICIENT MEn MJ O’Grady*, JL Tenover, P Getz*, J Grossman*, AM Lawson*, and SL Wolf*, Emory University, Atlanta, GA

OBJECTIVE: To determine if testosterone (T) supplementation combined with power training affects strength, function, or body composition in older androgen deficient men transitioning toward frailty. DESIGN: 6-month randomized placebo-controlled trial with 32 men assigned to one of four groups: 1) T and exercise, 2) placebo and exercise, 3) T with no exercise, and 4) placebo with no exercise. SUBJECTS: Thirty-two older (age 75.5 years) androgen deficient men (mean total T=299 ng/dl) transitioning toward frailty. INTERVENTION: Men received daily transdermal patches of either 10 mg T (Testoderm TTS) or placebo. Supplementation was designed to double the subject’s baseline T level. Twice weekly power weight-lifting targeted lower extremity muscle. Training volume was one set of eight repetitions with intensity of consecutive weekly sessions alternating between high, 80% one-repetition maximum (1-RM), at the beginning of the week and low; 60% 1-RM, with the final weekly session.

MEASURES: Dynamic isotonic strength (1-RM), body composition and bone mineral density by dual energy X-ray absorptiometry, and timed performance tests were obtained before and after the treatment period.

RESULTS: Strength, lean body mass and physical performance improved in both exercise groups relative to non-exercisers (p<0.02). Men assigned to T and exercise demonstrated greater increase in strength (p<0.01), lean body mass (p<0.02), bone mineral density (p<0.01), and performance-based measures (p<0.03) than those assigned to the exercise and placebo. CONCLUSIONS: Power training combined with testosterone increases strength, lean body mass, bone mineral density and function more than achieved with either intervention alone in older androgen deficient men in transition toward frailty.

P5/6 – 083

THE MOLECULAR BIOLOGY AND PATHOLOGY OF THE AGING RAT VENTRAL PROSTATE John Scolaro*, Colm Morrissey*, Adam Buser*, Jacintha O’Sullivan*, Amy Moquin* and Martin Tenniswood, Department of Biological Sciences, University of Notre Dame, IN 46556 and The Trudeau Institute, Saranac Lake, NY 12983*

The rat ventral prostate is composed primarily of hormonally dependent tall columnar secretory epithelial cells and cells of stromal origin. Androgen ablation causes the gland to regress. We have observed differences in the morphology and rate of regression of the ventral prostate in 3 and 12 month-old Sprague Dawley rats before and after castration. The intact 3 month-old rat ventral prostate contains tall columnar secretory epithelial cells that line the lumen and make up approximately 80% of the tissue. After castration, the gland regresses within 6 days, as the androgen dependent epithelial cells undergo apoptosis. In contrast the number of tall columnar epithelial cells in the intact 12 month-old rat ventral prostate is significantly diminished even before castration and continues to regress up to 12 days after castration. The ventral prostates from intact 12 month-old animals also contain autofluorescent bodies which we have characterized using electron microscopy, oil red, Sudan black and periodic acid Schiff’s staining as lipofuscin granules. These granules are retained in the gland after regression. Since lipofuscin is associated with oxidative stress and metabolism, we have compared the steady state mRNA levels of enzymes involved in the anti-oxidant enzyme system using RT-PCR analysis between 3 and 12 month-old animals. These studies are currently being confirmed using laser capture microscopy RT-PCR. These observations suggest the 12 month-old rat ventral prostate may be a more pertinent model for the aging human prostate than the 3 month-old rat ventral prostate. (Supported by USPHS CA69233 and the Coleman Foundation)
P5/6 – 084

SUPRAPHYSIOLOGIC ANDROGEN ADMINISTRATION IN ELDERLY MEN UNDERGOING JOINT REPLACEMENT SURGERY. [K Amory,† HC Chansky‡*, BD Anawalt,† AM Matsumoto,† WJ Bremner*, †Medicine, ‡Orthopedics, †Geriatrics, †Veterans Affairs-Puget Sound Health Care System, Seattle, WA and †Medicine, University of Washington, Seattle, WA

Introduction: Elderly patients undergoing joint replacement surgery can require extended rehabilitation. Supraphysiologic androgen administration to normal young men results in exercise-independent increases in strength without adverse effects. We hypothesized that short-term, pre-op supraphysiologic androgen therapy would improve strength and post-operative outcomes for elderly males undergoing joint replacement surgery.

Methods: We enrolled 36 elderly men (mean age 70.5 yrs.) in a double-blind, placebo-controlled trial of pre-operative, supraphysiologic (600mg testosterone enanthate (TE) IM/weekly x 4 weeks) or sesame oil placebo on outcomes of joint replacement surgery. We compared length of hospital stay and functional recovery using the FIM, a validated 7 point scale of disability. In addition, we assessed changes in testosterone and hematocrit. Statistical comparisons were made using ANOVA.

Results: (mean ± SD) Post-op FIM-After 3 weeks TE
Patients (number) Post-op stay FIM- Standing walk Serum T Hematocrit (da.y) FIM-walk (mol/L) (%)
TE (18) 5.8±2.1 5.5±1.0 5.1±1.5 89±18 45±5.7
Placebo (14) 6.6±2.4 4.5±1.0 4.2±1.2 124±8 41±3.8
p-value
0.31 0.06 0.21 <0.0001 0.01

There were no complications attributable to TE administration. Conclusions: In elderly men undergoing joint replacement surgery, pre-operative supraphysiologic androgen administration is safe, but without substantial clinical benefit.

P5/6 – 085

Androgeonne counselling in Eastern Hungary

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Significant hormonal changes do not only occur in women around menopause, but also in men of similar agegroup around andropause.

Little attention is paid to these physiologic changes of andropause by the general medical community. This is one of the main reasons, why most patients only receive symptomal treatment, where more effective hormone replacement therapy (HRT) is also available. Many complaints end up being treated by internists and psychiatrists without the added specialised counselling and care by andrologist specialists.

In the last five years, all patients presenting at the Andrology Counselling Service at Kenézy Gyula County Hospital, Hungary, underwent extensive internal medical, urological, endocrinological and andrological evaluation before commencement of HRT in conjunction with adjuvant therapy. Our experience, based on patient satisfaction on concurrent checkups, showed that these individuals regained their social stability thanks to HRT and individually suited adjuvant therapy.

P5/6 – 086

THE INCIDENCE FOR INTAKE OF ANIMAL PRODUCTS WITH PHARMACOLOGICAL ACTIVE RESIDUES OF ZERANOL AND ITS POSSIBLE EFFECT ON MALE FERTILITY.

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Introduction: Zeranol, a synthetic oestrogenic agent, is the most commonly used growth promoter in the United States of America. The use of growth hormones in food producing animals increases the slaughterweight and improves the food conversion. If meat containing residues of zeranol is consumed by males it could have an effect on their fertility, due to its oestrogenic nature.

Method: The study samples were collected from abattoirs in different towns in the Free State. Liver, urine and bile from each animal were collected to estimate the zeranol residue in these samples. The Ridascreen® enzyme immunosay for the quantitative analysis of zeranol was used.

Results: No zeranol residues could be found in any of these samples even though some of the animals that were slaughtered were implanted with zeranol.

Conclusion: The meat consumed in the Free State will probably have no effect on the fertility of males in this region, as no residues of zeranol could be found in these samples.

P5/6 – 087

THE EPIDIDYMIS: A TOXICOLOGICAL ANALOG OF THE KIDNEY?

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The epididymis and kidney arise from the same embryonic (mesonephric) tissue and share functional characteristics, such as the ability to modify luminal contents and establish solute gradients. Metabolic activation is important in the nephrotoxicity of some compounds, and it is possible that epididymal toxicity could arise by similar mechanisms. To test this hypothesis, we investigated whether the rat epididymis contains metabolizing enzymes typically found in the kidney. One such enzyme, cytochrome P450 2E1, metabolizes chlorinated solvents to toxic products in the kidney. In the present study, cytochrome P450 2E1 was immunoclo- nalized in the efferent ducts and epididymis of the rat: tissues were dissected from untreated rats, immersion-fixed in 1% paraformaldehyde, and frozen embedded. Tissue sections (12 μm) were probed with anti-rat cytochrome P450 2E1 and AlexaFluor488 conjugated IgC, and visualized using a fluorescent Olympus Provis microscope. The efferent ducts contained cytochrome P450 2E1 at levels comparable to that seen in the renal proximal convoluted tubules and liver centrilobular region. The caput, corpus, and cauda contained a qualitatively lower signal, while no signal was found in the initial segment. The presence of cytochrome P450 2E1 in the efferent ducts was confirmed by Western blotting using liver microsomes as a positive control. Metabolic activation of chlorinated solvents is associated with formation of a dichloroacetyl adduct in the liver and kidney. Our preliminary data indicate dichloroacetyl adduct formation in the liver centrilobular region of trichloroethylene-treated rats. Present studies are aimed at immunolocalizing this adduct in the kidney and epididymis of dosed animals and comparing the results to the regional localization of cytochrome P450 2E1.
P5/6 - 088

THE EFFECT OF CHRONIC SMOKING ON SEMINAL PLASMA INSULIN-LIKE GROWTH FACTOR-1 IN IDIOPATHIC OLIGO-ASTHENO-TERATOZOOSPERMIA (OAT SYNDROME).

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The objective of this study was to evaluate the effect of chronic smoking on seminal plasma IGF-1 & its hazards on semen quality in idiopathic OAT syndrome. This study included 9 normozoospermic fertile non-smokers, 10 fertile smokers and 9 infertile smokers with OAT for unknown etiology. Exclusion of other possible causes was done during selection of cases. Full clinical & scrotal ultrasonographic evaluations were followed by semen analysis. Seminal cotinine & IGF-1 were detected by radioimmunoassay technique. A significant decrease in seminal IGF-1 levels was detected in both smoker groups (fertile & infertile with OAT) to be 55.3, 39.88 ng/ml respectively, compared to fertile non-smokers (115.66ng/ml). Fertile smokers showed significant decrease in sperm production index, % of motile & rapid linear progressive sperm compared to non-smokers with increase abnormal forms. There was significant negative correlation of seminal cotinine level with seminal IGF-1, %motile & normal forms of sperm in smokers with OAT. Also, in this group, a positive correlation of seminal IGF-1 was detected with % motile & rapid linear Progressive sperm. Using multiple regression analysis, seminal cotinine & IGF-1 were found to be significant independent factors for prediction of certain sperm parameters (density, motility & abnormal forms) in OAT syndrome.

In conclusion, nicotine and its metabolites decrease IGF-1 secretion in semen adding to its hazard effects on spermatogenesis & sperm kinetic parameters.

P5/6 - 089

CONTRIBUTION OF ENVIRONMENTAL FACTORS TO THE RISK OF MALE INFERTILITY.

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An increasing number of reports suggest that chemical and physical agents in the environment, introduced and spread by human activity, may affect male fertility in humans. We investigated the relationships between exposure to environmental agents and seminal characteristics, and levels of reproductive hormones in the serum of men seeking infertility treatment. We studied 253 male partners from consecutive couples, who had their first infertility consultation between 1995 and 1998, in the Litoral Sur region of Argentina, one of the most productive farming regions in the world. A multivariate logistic regression model, adjusted for confounding factors, showed that exposure to pesticides and solvents is significantly associated with sperm threshold values well below the limit for male fertility. We also found that men exposed to pesticides had higher serum estradiol levels, and that men exposed to solvents had lower levels of luteinizing hormone than non-exposed men. All these effects were greater in men with primary infertility than in men with secondary infertility. In conclusion, we show that environmental factors contribute to the severity of infertility, which suggest that these factors may worsen the effects of pre-existing genetic or medical risk factors.

P5/6 - 090

TIME COURSE EFFECTS OF DIETHYLSTILBESTROL (DES) ON FERTILITY OF ADULT MALE RATS.

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We previously reported that DES at a rate of 8 µg/rat/day for 12 days altered epithidymal sperm functions, but with minimal or no alterations in sperm production and morphology. The present study evaluated the time course effects of DES on male fertility. Rats (5/group) received DES at a rate of 8 µg/rat/day for 6, 9, or 12 days, respectively. The control group (3/group) received corn oil. At the end of each treatment, each male was cohabited with two untreated females for 12 days. Fertility-related parameters studied were 1) fertility index (number of males that impregnated at least one female/number of cohabitant males x 100), 2) potency or pregnancy index (number of pregnant females/number of cohabitant females x 100), 3) number of pups per litter, and 4) presence of copulatory plugs. While fertility and potency indices were (100% and 94%), respectively, in controls, these were reduced to 60% each at day 6, 40% and 30%, respectively, at day 9, and 0% each at day 12 of the treatment. Copulatory plugs were observed in 16/18 control females, in contrast to 2/10 in the 6-day and U/10 each in the 9- and 12-day groups. The number of pups per litter ranged from 12-16 in controls, 3-10 in the 6-day group, and 8-15 in the 9-day group. Results indicate that 1) DES affects fertility in a time-dependent manner and 2) reductions in fertility parameters can be seen as early as 6 days of DES treatment. The DES-induced reductions in fertility may result from deficits in epididymal sperm functions and/or a compromise in sexual behavior (as evident from lack of copulatory plugs). Supported by NIH/MBRS S 506-GM-08091 grant.

P5/6 - 091

SEROTILI CELL INJURY IN ETHANOL-TREATED RATS: NOVEL ROLES FOR APOPTOSIS-RELATED GENES


Chronic alcoholism is associated with testicular damage and marked germ cell loss in human and experimental animals. However, the nature and molecular mechanisms controlling such loss are not yet clearly understood. Here we hypothesized that exacerbated apoptosis of germ cells secondary to Sertoli cell injury may be a possible mechanism for ethanol-induced germ cell loss. Adult Wistar rats were fed either Liber De Carlie liquid diet (5% ethanol) or an isocaloric control diet for 12 weeks. Morphological features of Sertoli cell injury as basal vacuolization, lipid accumulation, germ cell pyknosis and detachment, giant cell formation and abnormal Sertoli cell nuclei were observed in the seminiferous tubules (ST) of ethanol-treated rats (ETR) under both light and electron microscope. TUNEL staining demonstrated marked increase in the number of apoptotic germ cells in ETR compared to control group. Increased expression of FasL in Sertoli cells and Fas in germ cells of ETR compared to a basal lower level of expression in control ST was detected immunohistochemically and was supported by RT-PCR. Over-expression of p53 in most types of germ cells and some Sertoli cells and induction of iNOS in Sertoli cells and myoid cells were detected in ST of ETR using immunohistochemistry. Moreover, caspase-3 expression was increased in germ cells of ETR. It seems that the apoptotic effect of ethanol on ST is mainly secondary to Sertoli cell injury caused by increased oxidative stress, evidenced by induction and upregulation of iNOS. This results in the upregulation of FasL in Sertoli cells with subsequent p53-mediated Fas upregulation in germ cells leading to caspase-3 activation and germ cell apoptosis that may be involved in infertility associated with alcoholism.
P5/6 – 092
SCREENING AND TESTING METHOD FOR ENDOCRINE DISRUPTORS - RODENT 20-DAY THYROID/PUBERAL MALE ASSAY
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To establish the 20-day thyroid/puberal male assay, we dosed intact male SD rats from postnatal day (PND) 33 to 53 with testosterone, diethylstilbestrol (DES), flutamide and ketoconazole as test chemicals, and examined several reproductive endpoints. Preputial separation (PPS) was significantly delayed in the DES (20 and 40 ug/kg) and flutamide (5 and 25 mg). The male rats were killed on PND 53 or 54, and pituitary, thyroid, and male reproductive organs were removed and weighed. DES and flutamide treatment resulted in a significant reduction in the weights of epidiymes, ventral prostate, seminal vesicles, levator ani bulbocavernous muscles (LABC), Cowper’s glands, and glans penis. Ketoconazole treatment resulted in a significant increase in seminal vesicles and glans penis. Testes weights were unaffected in all treatment groups. Serum testosterone decreased significantly in DES and ketoconazole treatment group. No differences were observed in thyroxine (T4) level between the treatment groups and the control. To evaluate whether phospholipid hydroperoxide glutathione peroxidase (PHGPx) can be useful for screening endocrine disruptors, we examined the expression of its mRNA, PHGPx expression was increased significantly in testis, epididymis, ventral prostates, seminal vesicles, and LABC of DES, flutamide and ketoconazole treatment group. These results indicate that DES and flutamide delays puberty in the male rat and its mode of action appears to be altering the secretion of steroids and having subsequent effects on the development of the reproductive tract. Thus, this assay can be used as an alternative method for screening antiandrogen activities, but further studies are necessary to investigate the reliability of this assay. PHGPx may be a useful biomarker to detect endocrine disrupting chemicals.

P5/6 – 093
RNA SYNTHESIS IN HUMAN TESTIS: INTERFERENCE BY PARATHION.

A wide set of genes is expressed during spermatogenesis. Some of them are transcribed solely in germ cells, associated to typical meiotic patterns, to the structural reorganization of DNA, or with synthesis and storage of RNA, from preleptotene spermatocytes up to late pachytene. Massive use of agropesticides has elicited interest on potential adverse effects on human and animal reproductive health. Exposure for long periods even at low doses could induce subclinical, epigenetic or genetic effects. This work deals with the evaluation of the in vitro effect of Parathion on RNA synthesis by human testicular tissue. The pesticide was used at concentrations similar to those found in agroproducts. Six semen human testis were used after medically indicated orchectomy. Tissue samples of 12 mg each were used in triplicate in all experiments. Incubation was done for 4 hours at decreasing concentration of Parathion (0.8; 0.4; 0.04; 0.0004 and 0.0004 µM, and control). Tritiated Uridine (SpCi) was added for one hour. Thereafter, total RNA and Uridine uptake were measured to determine an inhibited fraction (IF: Cpm/µg). In other experiments, control and 0.8 µM parathion triplicate samples were incubated for 5, 6, 7 and 8 hrs. After the 5th hr (and every hour thereafter) the incubation medium was renewed, free from pesticide. In each group H3-Uridine was added for one hour after the 5th, 6th, 7th or 8th hr, respectively. A linear regression (γ = -0.02286 + 0.005456X) was found parathion at a concentration of 0.8µM decreases. RNA synthesis (IF 0.37) and the effect is dose dependent. It is reversible when the pesticide is eliminated from the medium (IF 0.23 till no effect). Therefore, organophosphorous agropesticides at low concentration affect spermatogenesis by interfering with RNA synthesis.

P5/6 – 094
REPRODUCTIVE TOXICOLOGY OF ETHANOL IN MALE RATS.
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Abuse consumption of ethanol is one of the most significant health and social problems, especially among youth. The effects on male reproductive function in humans include reduction of sexual interest, ejaculation problems, and sterility. The objective of this work was to evaluate reproductive parameters such as sexual behavior, fertility after natural mating, organ weights, and germ-cell morphology and counts in adult male rats exposed to ethanol since puberty. 30 male Wistar rats, 50 days old, were divided into experimental groups of 10 rats each: G1, which received a liquid diet with 36% of the daily calories derived from ethanol and the remainder from Sustacal/E (Mead Johnson); G2, similar to G1, but with an isocaloric amount of sucrose in substitution for ethanol; G3, which received commercial lab chow. All groups had free access to filtered water. After 55 days of treatment (one spermatogenic cycle), animals were tested for sexual behavior. Just 33% of G1 rats performed mounts when paired with receptive females, and only 22% of those reached ejaculation, while 50% of G2 and 70% of G3 did the same. After natural mating, the fertility potential of G1 was significantly reduced to 16% when compared to G2 = 91% and G3 = 92%. A significant reduction was also observed in body, testis, epididymis, and vas deferens weights among ethanol treated rats. The daily sperm production and the sperm counts in the epididymis was also significantly diminished after ethanol treatment. Sperm with abnormal shape were significantly increased in G1. The results suggest that chronic consumption of ethanol provokes adverse effects on the male reproductive function of male rats, altering important parameters.

P5/6 – 095
POSSIBLE ROLE OF REACTIVE OXYGEN SPECIES ON HUMAN SPERM QUALITY AND FUNCTION
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Sperm capacitation involving intracellular and membrane changes is necessary for fertilization of oocytes. Reactive oxygen species may exert both toxic and physiological regulating action on spermatozoa. This present work was designed to examine the occurrence of malondialdehyde (MDA) in sperm suspension, and evaluate its influence on sperm quality and in vitro fertilization capacity. A total of 67 male patients attending conventional IVF treatment programs were included in this study. Washed spermatozoa were pre-incubated at 37°C in 1:4T2 for 3h before IVF procedure. Sperm motility, viability determined by hypoxic-motic swelling test and MDA determined by thiobarbituric acid method were analyzed; fertilization was examined 18-20h after insemination. The patients were divided into two groups on the basis of fertilization rate. In group A(n=52), fertilization rates were higher than 70% (normal), whereas in group B (n=15) fertilization rates were lower than 10% (fertility failure). The value of group A were compared with the respective value of group B. Significant decreases in percentage of motile and swelling spermatozoa in group B were observed in comparison with group A (P<0.01). However, the MDA concentration of group B was remarkably higher than that of group A (P<0.01). These results demonstrate that the presence of MDA may be relate to decrease of human sperm quality and fertilization, and the measurement of MDA can provide important information on the spermatozoa quality and the clinic diagnosis of male infertility and should be of value in the optimization of some IVF treatment procedure via ICSI.
Abstracts – Poster Session 5/6

P5/6 - 096

POLYCHLORINATED BIPHENYLS (AROCLOD 1254) TREATMENT CAUSES ASPERMIA IN RHESUS MONKEY (MACACA MULATTA) 
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Polychlorinated biphenyls (PCBs) are the environmental toxicants that persist in the global ecosystem and accumulate in trophic hierarchy. These chemicals are found to be carcinogenic and teratogenic, disrupt reproduction and endocrine functions besides exhibiting hepatotoxicity and dermal toxicity. Adult male rhesus monkeys (Macaca mulatta) were given oral treatment (n=4 each) of either Aroclo 1254 at a dose of 200 μg/kg/day/animal or vehicle (corn oil and glycerol) for six months. During treatment period body weights and testicular size were noted and blood samples (3 ml) were collected on weekly basis. At the end of treatment period animals were sacrificed humanely. Testis and accessory glands (epididymides, seminal vesicles and prostates) were removed and fixed for microscopy. Serum testosterone concentration was determined by radioimmunoassay. Body weights and testicular diameter of Aroclo 1254 treated animals decreased significantly during the treatment period. Aroclo 1254 treatment could not decline testosterone levels in the testes and accessory organs. The seminiferous tubules were characterized by the cessation in spermatogenetic activity. Spermatagonia were very less and mostly abnormally large in size. The Sertoli cells were widespread in the cords but seemed shrunken and contained fat droplets. Leydig cells were normal, scattered randomly in the interstitium. The basement membrane of seminiferous cords appeared thickened. In epididymides, the luminal spaces were much reduced in size and lacked spermatozoon. At some places lumens were completely absent and the epithelium was much thicker and stratified. In the prostate glands the lumens were either collapsed or much reduced in area and lined with a single layer of cells that had lost their normal cuboidal/squamous shape due to shrinkage. The connective tissue between acini was increased. Seminal vesicles too were damaged by PCB treatment. It is concluded that Aroclo 1254 has a direct toxic effect on gonads and accessory tissues and its effects are not mediated through down regulation of testosterone.

P5/6 - 098

LEAD AND CADMIUM CONCENTRATIONS IN SEMINAL PLASMA AND COMPARISON WITH SEMINAL QUALITY IN HEALTHY KOREAN ARMY RECRUITS. 
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A prospective study on semen analysis of healthy young Korean male was conducted, and the seminal lead and cadmium which are known to affect endocrine system were also measured and compared with various parameters from the semen analysis and the comprehensive questionnaire. Cadmium and lead concentrations in the seminal plasma of 150 healthy army recruits in September and November, 2000 were measured by inductively coupled mass spectrometry. The relationship between metal concentration and parameters of semen analysis was studied. The mean cadmium and lead levels in seminal plasma was 1.165±1.321 μg/ml and 5.996±4.737 μg/ml, respectively, which is comparable to the data of the other countries. These heavy metals seem to be correlated coffee drinking (p=0.0308) and solvent use (p=0.0310) with statistical significance, however the small number of sample size preclude from drawing any conclusive remarks. Mean sperm concentration was 74.14 x 10^6/ml and the seminal parameters were unrelated to the various factors in the comprehensive questionnaire.

P5/6 - 097

PESTICIDE EXPOSURE AND FERTILITY: RESULTS OF AN EPIDEMIOLOGICAL STUDY OF FRENCH VINEYARD WORKERS. 
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Design: In 1996, we conducted an epidemiological study on the vineyard workers of Alsace to investigate the effects of pesticides on human fertility. Occupational physicians asked workers to participate i) in the retrospective part of the study, by completing a questionnaire with their wives ii) in the prospective part of the study, by providing hair and sperm samples before and after the pesticide spraying season. The pesticide exposed and non-exposed groups were compared by using the discrete Cox model (adjusted for confounder ratios). Student's paired t-test and the Wilcoxon paired test were used to compare the sperm characteristics before and after the pesticide spraying season. Results: In the retrospective study, the adjusted confounding ratio for pesticide exposure did not differ significantly from 1 (1.25 (95% CI: 0.84 - 1.86)). Sperm density was unaffected with a mean of 79.1 million sperm per ml of ejaculate before the pesticide spraying season and 76.7 million at the end of the pesticide spraying season (n.s.). There was no change during the pesticide spraying season in sperm motility (53.0% before versus 50.3% after), and in total sperm count (261.0 million before versus 217.9 after).
P5/6 – 100

FAS SIGNALING SYSTEM MEDIATES CADMIUM-INDUCED TESTICULAR APOPTOSIS IN RATS. THE POSSIBLE PROTECTIVE ROLE OF SELENIUM.

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Apoptosis occurs in the testis as an important physiological mechanism to limit the number of germ cells in the seminiferous epithelium. Fas system was considered as a key regulator of spermatogenesis. Fas, a transmembrane receptor protein, transmits an apoptotic signal within cells when bound to Fas ligand (FasL). The involvement of Fas system in germ cell apoptosis was assessed. This study was designed to examine whether the Fas/FasL system is involved in mediating testicular tissue apoptosis in male rats by using cadmium (Cd) an environmental pollutant as a testicular injury model. The possibility that selenium supplementation might prevent these testicular effects was also examined. Rats were treated with CdCl2 (0.5 mg/kg, orally, daily for 5 weeks). The typically apoptotic DNA fragmentation and the associated testicular histopathologic changes that are induced by Cd treatment were suppressed by pretreatment of rats with sodium selenite (1 mg/kg, i.p., daily for 5 weeks). Cadmium increased the expression of mRNA of FasL (by RT-PCR) in rat testis as well as revealed an increase in the numbers of Fas positive germ cells (by immunohistoch- emistry). This up-regulation of Fas/FasL expression in the testis was suppressed by selenite treatment. These results indicate that (1) Chronic cadmium exposure induced germ cell apoptosis in rats that may lead to suppression of spermatogenesis and infertility; (2) the up-regulated Fas signaling system is involved in stimulating this testicular apoptosis; (3) selenium supplement was effective in preventing cadmium induced testicular apoptosis.

P5/6 – 102

EFFECTS OF CASTRATION ON LINDANE MEDIATED HEPATO-TOXICITY.

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Lindane, a commonly used chlorinated insecticide, is a persistent environmental contaminant. In the non-target mammalian species, lindane produces severe hepato, neuro and reproductive toxicity. No reports are available on the role of sex hormone insufficiencies in lindane mediated effects on hepatic drug metabolizing enzymes and oxidative stress in male rats. To answer this question male rats of 100-150 g bwt were surgically castrated. Fourteen days after the surgeries the animals were administered lindane (100 mg/kg bwt x 6 days) intraperitoneally. The results indicated that cytochrome P450 content of liver was elevated significantly following lindane treatment to castrated (p<0.001) and non-castrated (p<0.01) animals. This effect, therefore, was pronounced in case of castrated rats. Similar effects were observed when the lipid peroxidative indices were measured in terms of thiobarbituric acid reactive substances. The animals (castrated/non-castrated) remained refractory as far as hepatic non-protein thiol content. The activity of glutathione-S-transferase declined sharply in lindane treated castrated animals compared to its respective controls or lindane treated non-castrated animals. These results show that testosterone deficiency caused by castration augments lindane mediated hepatotoxicity. Further, these results imply that the individuals with testicular dysfunction leading to testosterone insufficiency might be at greater risk of liver toxicity following accidental or occupational exposure to lindane.

P5/6 – 103

EFFECTS OF IN UTERO EXPOSURE TO TRIBUTYLtin ON SPERM MOTILITY IN ADULT RATS

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TrIBUTYLTin (TBT) is an environmental contaminant commonly used in anti-fouling agents for boats, as well as a by-product from several industrial processes. TBT has been shown to be immunotox- ic, however, little information exists regarding the effects of TBT on mammalian reproduction. The objective of this study was to determine the effects of TBT on sperm motility, in male offspring exposed in utero to TBT. Female Sprague-Dawley rats were impreg- nated and exposed to TBT (0, 2.5, 10, 20 mg/kg) by gavage throughout gestation. The pups were weaned on day 21 and sampled at day 91 for sperm analyses using an IVOS sperm analyzer. There were 6 animals per group with the exception of the high dose where only 2 animals survived treatment. Body weights of TBT treated rats were 10 and 30% lower than controls in the 10 and 20mg/kg groups respectively. While tests weights were slightly decreased by TBT, these differences were not significant. Sperm from the cauda epididymis were removed and were assessed for a variety of parameters including motility, concentration, and progressive velocity. Sperm motility was decreased by 15% in the 10mg/kg group and 21% in the 20 mg/kg. Average track speed of the sperm was also increased in the treated groups. There were no significant differences in other parameters such as beat frequency and lateral amplitude. These results suggest that exposure to TBT in utero can result in decreased sperm function when the animal reaches adulthood. Supported by the Toxic Substances Research Initiative.

Abstracts – Poster Session 5/6

P5/6 – 101

ENVIRONMENTAL POLLUTION AFFECTS NUMBER, VIABILITY AND MOTILITY OF SPERMATOZOA: A STUDY BY C.A.S.A.

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It is well known that environmental chemical pollutants act on several organs and, particularly, on testicular function. The aim of this study was to evaluate the sperm function of 150 workers (age 26-62 years) at toll payment station of a motorway, representing a peculiar group of subjects exposed to environmental pollutants. They were studied evaluating sperm count, motility and viability and plasma levels of methemoglobin (MHB), sulphohemoglobin (SHb), Pb, Zn-protoporphyrin (ZnPP). The sperm motility was studied by C.A.S.A. evaluating VSL, VCL, LIN, ALH. The semen patterns were compared with those of 60 normal controls. The statisti- cal analyses was performed by linear correlation between the semen patterns and environmental pollutants and plasma marks- ers of toxic occupational exposure. The sperm count and viability were inversely correlated with Pb plasma levels (p<0.005). Total motility, LIN and ALH were inversely correlated with MHB plasma levels (p<0.005). In conclusion, we could affirm that environ- mental pollution induces an impairment of sperm parameters, above all, as it regards count, motility and viability.
P5/6 – 104
EFFECTS OF DIOXIN ON PENILE ERECTION IN THE RABBIT.
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Introduction and Objectives: Recently, dioxin (TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin) has received much attention worldwide as an endocrine disruptor. Decreases in spermatogenesis and the ability to conceive and carry a pregnancy to term are the most sensitive signs of reproductive toxicity by TCDD in the mammal but no report of its effect on erectile function exists. We performed this study to investigate the effect of dioxin on the erectile function. Methods: In four groups of New Zealand white rabbits, Group I: Control, II: 4 weeks after 1ug/kg of TCDD (i.p.), III: 8 weeks after 1g/kg of TCDD, cavernosal tissues strips were harvested for pharmacological study in the organ bath. Isometric tension was established with phenylephrine (10-6M). Contractions were studied with noradrenaline and relaxations were studied with acetylcholine and sodium nitroprusside. In a separate experiment, histological examination was performed in the animals of corresponding groups. Results: Compared to Group I, the contractions and relaxations of cavernosal strips were significantly suppressed in Group II and III (<p<0.01). In Group III, even higher concentration (10-3M) of acetylcholine did not relax the precontracted strips. Histologically, thickening of tunica albuginea with markedly increased collagen fibers, subcutaneous deposition of fat, decreased sinnoidal space by severe cavernosal smooth muscle hyperplasia and cavernosal fibrosis were identified in all TCDD-treated animals. Conclusions: These results suggest that dioxin leads erectile dysfunction via penile histologic changes in rabbit. On following studies with low dose of TCDD, the effects on rabbit penis and human penis should be identified to determine whether TCDD induces erectile dysfunction in human.

P5/6 – 105
EFFECT OF IN UTERO EXPOSURE TO TRIBUTYL Tin ON FETAL RAT TESTIS.
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Tributyltin (TBT) is used as an antifouling agent as a biocide in agriculture and as a heat stabilizer. In this study we investigate the adverse effects of TBT on fetal rat. Pregnant rats were treated with doses of TBT 0.25, 2.5, 10 and 20 mg/kg body weight, by a gastric gavage from gestation day 0 -19. Another group of animals was treated with the same doses from gestation day 8-19. The mothers were killed on day 20 and fetal testes were removed, fixed in Bouin's or glutaraldehyde and embedded in paraffin or Epon respectively. Paraffin sections were either stained routinely with H&E for light microscopy (LM) analysis or immunostained with the 3β-HSD antibody. Epon thick sections of testes were stained with toluidine blue while thin sections were stained with uranyl acetate and lead citrate for electron microscopy (EM). In the LM of the testis more degenerating gonocytes were noticed in the seminiferous tubules of the group treated with TBT, particularly at the 10 mg/kg dose, as compared to controls. Data also showed significant decrease of gonocyte number/unit area and an increase of Sertoli cells/gonocyte in the group treated with 10 mg of TBT at gestation day 8-19. The number of Leydig cells/unit area in the group treated with TBT (20 mg/kg) showed a decrease in comparison with controls. EM of fetal testes from TBT-treated animals (10 mg/kg, 0-19) revealed abnormally contorted nuclear and plasma membranes in gonocytes. Enlarged intercellular spaces between gonocytes and Sertoli cells were also observed in the group treated with 10 mg/kg of TBT. These results indicate that TBT adversely affects the number and morphology of germ cells and Leydig cells in fetal testis that may affect spermatogenesis (Supported by TSRI-58).

P5/6 – 106
EFFECT OF IN UTERO EXPOSURE TO ORGANO-CHLORINES AND POLYCHLORINATED BIPHENOLS ON THE RAT FETAL TESTIS.
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Persistent organic pollutants (POPs) such as organochlorines and polychlorinated biphenols are widespread contaminants of human and animal foods. Little is known about the effects of in utero exposure to POPs on the development of reproductive organs. The objective of this study is to assess the effects of a mixture of POPs in ratios found in the environment on the fetal rat testis. Pregnant Sprague Dawley rats were treated by gastric gavage with a vehicle or a mixture of POPs, identified as components of the diet of inuits living on Broughton Island (10, 100 or 1000 times dietary exposure). One group of pregnant females was treated from gestation day 0-19 and the second group from gestation day 8-19. The mothers were killed at day 20. Testes were removed from the fetuses and fixed in Bouin's or glutaraldehyde and embedded in paraffin or Epon respectively. Paraffin sections were stained routinely with hematoxylin and eosin for LM or immunostained with the 3β-HSD antibody. Epon sections were stained with toluidine blue. The results showed increased Sertoli cell and reduced gonocyte numbers with increasing doses of POPs. However, these values were not significant. When the values were expressed as the ratio of Sertoli cells/gonocyte, there was a significant increase (39%) in the group exposed to X1000 (day 0-19). The number of Leydig cells/unit area did not change in both groups. These results suggest that exposure to POPs decreases the number of gonocytes in the fetal testis. (Supported by TSRI-152)

P5/6 – 107
CYTOTOXIC EFFECTS OF ORGANOPHOSPHORIC AGROPESTICIDES ON BOVINE AND PORCINE SEMEN.
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Organophosphoric agropesticides (OP) are still widely used. Farm animals are potentially exposed by grazing in contaminated areas. Therefore, analysis of the effect of Parathion* (PT) and its metabolite, paraoxon (PO), tested in vitro, upon seminal parameters of bull and pig sperm was done. Frozen semen of bull (12 samples) and pig (21 samples) used for insemination, were thawed and incubated with pure Parathion* (98%) or paraoxon (90%) at concentrations of 0,05; 0,1; 0,2; 0,4; and 0,8 mM. Conventional semen analysis plus HOS-test, acrosome reaction (AR; triple staining) and chromatin decondensation (SDS/DTT) were performed (CD).

Results indicated plasma membrane damage with high PT concentrations (but not with PO) and decreased vitality in bulk sperm. AR and CD were not affected. In pig's semen, vitality of spermatozoa was altered only by PT at 0,8 mM but not by PO. No AR was elicited but false AR was found in dead porcine sperm. Plasma membrane function was affected by both PT and PO, at concentrations of 0,4 and 0,8 mM. CD was increased in a dose dependent fashion both with PT and PO. The most affected parameter was membrane integrity and consequently, sperm vitality. This may be due to the lipophilic properties of OP. Acrosomic membranes, however, behave differently in bull than in pig. Nuclear CD was not affected in the bull. Both OP are highly electrophilic and could interact with disulphur bonds of nuclear protein. Bull sperm (but not pig) contain only protamine I which is rich in cysteine and is thus resistant to CD.

Compromise of the three functional seminal parameters tested here are different for different animal species when exposed to OP; these pesticides have been also proved to alter human semen. (Contreras et al, 1999).
P5/6 – 108

EFFICACY OF SILDENAFIL VS INJECTION THERAPY FOR PSYCHOGENIC ERECTILE DYSFUNCTION (ED)CASES
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Objectives: Performance anxiety is the most frequent psychological situations encountered in psychogenic ED. It is often associated with an initial sexual encounter which is particularly evident in countries with restricted sexual relations e.g. Egypt and destroys a lot of early marriage cases, the psychotherapeutic lines are poorly accepted by those patients. The aim of this study is to compare injection therapy and sildenafil in management of those cases. Materials and methods: 150 recently married men who failed to do intercourse with their wives for more than one month of their marriage and up to two years and tried different medical treatments known to be taken in these situations. 50% of patients are subjected to injection therapy and then allowed to do the intercourse with their wives; the second half are prescribed sildenafil 100 mg and asked to start foreplay and intercourse two hours after. Both groups followed up on the next visit. Results: Injection group: 69% had erection after one injection which was sustained >2hours, 20% needed 2nd injections to get a sufficient erection for intercourse and the rest failed after 3 doses. On the follow up, patients of 1st two groups did their 1st intercourse very successfully and continued to behave normally later on, 3 patients from the 3rd group obtained an erection a few hours after injections but behave normally later on while the rest proved to have organic causes. At 6month follow up for our patients we found no more dysfunctions. B: Oral group: only 27% who succeeded to do their first intercourse 2 hours after oral administration, while the rest failed and shifted to injection therapy. Discussion: By interpretation we can say that injection therapy once or twice in psychogenic ED is very effective and superior to Viagra to treat those patients and rescue those families. Although trying Viagra first is advised.

P5/6 – 110

SILDENAFIL MONOTHERAPY VERSUS POLYPHARMACOTHERAPY FOR ERECTILE DYSFUNCTION
Dr.Vipan Bhatia*, Department Urology & Andrology, Oasis Hospital, Al Ain, United Arab Emirates

Introduction: Oral Sildenafil therapy is now a first line therapeutic option for the management of most patients with erectile dysfunction (ED). Recently alpha 1 blockers have also been postulated to improve the erectile and ejaculatory functions. ED and OOS(outflow obstructive symptoms) continue to be a common malady in males after the 5th decade. Objective: This study attempts to compare the efficacy of combined usage of Sildenafil and Alfuzosin versus Sildenafil alone in males with ED. Method & Methods: Patient population included 50 males, mean age 62 years (56-71 years). All patients had mild to moderate ED with a median duration of 1.6 years. None of the patients had significant OOS and the International Prostate Symptom Score was <12. post void residual urine on ultrasound <50 ml and peak flow rate >12 ml/sec PSA<ng/ml. All patients were subjected to detailed history taking, physical examination and “goal directed” ED evaluation. The obvious ED risk factors included – diabetes mellitus(22), hypertension(16), obesity(14) and smoking(14). Multiple risk factors existed in 26 patients. The patients were randomized into two therapeutic groups: Group A(25) Sildenafil 50mg once a hour before intercourse, twice a week. Group B(25) Sildenafil 50mg one hour before intercourse, twice a week + Alfuzosin 5mg twice a day continuously. The patients were evaluated at six weeks Results: The evaluation criteria used included: A) Question 3 of International Index of Erectile Function (IIEF)-ability to achieve erection, B) Question 4 of IIEFability to maintain erection, C) Global efficacy question (G EQ)- did this treatment improve your erection D) Partner satisfaction. The responses were graded on a scale of 1-5 (almost never-almost always). The data was subjected to statistical analysis. Full results will be discussed.

P5/6 – 109

ERECTILE DYSFUNCTION- ROLE OF SILDENAFIL SALVAGE THERAPY
Dr. Vipan Bhatia*, Department Of Urology & Andrology, Oasis Hospital, Al Ain, United Arab Emirates.

Introduction: Sildenafil is a potent selective inhibitor of phosphodiesterase type 5 is now accepted as a frontline oral pharmacotherapy for the majority of patients with erectile dysfunction (ED). Its therapeutic efficacy as a second line treatment for ED patients not responding to other widely prescribed modalities is not yet clearly established. Objectives: The aim of this study was to evaluate the role of Sildenafil in the management of ED refractory to other non operative therapeutic options. Material & Methods: The study group included 51 male patients, median age 63 years (range 46-79 years). The mean duration of ED was 2.7 years. All these patients were subjected to “goal oriented” management approach including detailed history, physical examination, appropriate laboratory or vascular evaluation as indicated. Recognized ED risk factors could be identified in 42 patients (82%) and included - diabetes mellitus (21), hypertension (19), obesity >90 kg (16), smoking (14), hyperlipidemia (12), azotemia (2) and hepatic cirrhosis (1). A combination of risk factors existed in 31 patients. The prior treatment options used by these patients included: intraurethral prostaglandin E1 (ICI-29), Intracavernous prostaglandin suppositories (IUS)-10, Vacuum erection devices (VES)-7, oral trazadone therapy-3 and oral apomorphine therapy-2. The mean period of usage was 3.6 months utilizing standard dosage and techniques. All the patients opted for Sildenafil due to unsatisfactory erectile response to the previous treatment options. The initial Sildenafil dose was 50mg one hour before sexual intercourse. In case of unsatisfactory response the dose was increased to 100 mg. Each patient was advised four attempts before documenting the results. Results: The response evaluation criteria used included: A) Question 3 of the International Index of Erectile Function (IIEF). Full results will be discussed.

P5/6 – 111

ERECTILE DYSFUNCTION POST RADICAL PELVIC SURGERY: MANAGEMENT WITH SILDENAFIL AND L-ARGININE EVALUATED BY BUCKLING- TEST
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OBJECTIVES: Radical pelvic surgery may develop sexual dysfunction of various degree but always possible to produce alterations of a somatopsychic equilibre of the person itself by preexistent factor at the 50%, age at a significant oncological risk and moreover at high incidence of benign pathology but however for others as diabetes and hypertension besides conditioning handicaps as vaso dilatation, grey growing, baldness and others, to which everyone, in silent sufferance, adjust a resigned survival.

MATERIAL AND METHODS: In the last 3 years, we performed 88 cystectomies with various urinary diversions and 146 radical prostatectomies. Excluding patients with heart disease, diabetics and uninterested, n=116 pts. were run in the study (64 prostatectomies and 52 cystectomies) with average age of 65. Oral administration of L-Arginine was four ampules (1g) daily for three months. L-Arginine HCI is the obligatory precursor of Nitric Oxide (NO) chemical mediator of erectile sinusal dilatation and therefore worthy helper of Sildenafil function that is effective just joint to NO. Furthermore, it has a promoting effect on sexual functions. Side effects of type V phosphodesinase. In physiological state cyclic Guanosin Monophosphate (cGMP) existing erectile relaxation, is disarranged just by the phosphodesinase that is itself inhibited by Sildenafil. Its administration was associated to visual sex stimulation and evaluated by Buckling-test (dynamometric validation of erection). Modern uroled surgery researches on penile hemodynamics, emphasize the role of three functions in the achievement of penile axial rigidity: intracavernous pressure, penile geometry and cavernous tissue features. Clinical application of those rules enabled to evaluate in mg. From 2 to 1000 (n. v. more than 500) the cavernous level of Buckling under a testing weight (Buckling-test). Patients were randomised in two groups: 1) Sildenafil 2) Sildenafil + L-Arginine.

RESULTS: The standose of 50 mg. was for everyone insufficient (Buckling test 0-250) whereas 100 mg. were effective for a significant number of patients: Buckling test more than 500 mg. in 19% of the first group and in 38% of the second group. The same administration, taken at home in more suitable sexual conditions, increased the results to 26% and 48% according to evaluation of a telephon interview.

CONCLUSIONS: Until now the only medical treatment at disposition for post surgical erectile dysfunction were PC1 1 injection. Today we believe that at least a Sildenafil test would be administered to those patients. Even if a restricted number of them can obtain a resolutive benefit from the management nevertheless it can offer the less invasive way for a sexual rehabilitation. Its early administration with L-Arginine seems to increase significantly through the reoxygencation, a functional restoration of erectile tissue.

VIIth International Congress of Andrology | 181
P5/6 – 112

ORAL SILDENAFIL IN THE TREATMENT OF ARTERIOGENIC IMPOTENT PATIENTS
M. Mancini*, L. Negrì*, Piediferro G* and GM Colpi*; Andrology Service, San Paolo Hospital, Milan, Italy

INTRODUCTION. About one third of men with erectile dysfunction are not fully satisfied with the performance of Sildenafil, taken when needed before sexual intercourse. In these cases we hypothesized atherosclerosis. Our purpose was to assess if a daily oral administration of Sildenafil could increase Peak Sistolic Velocity (PSV) of cavernosal arteries in arteriogenic impotent patients.

MATERIALS AND METHODS. Sixteen patients were randomized to receive daily placebo (N=8) or sildenafil 25mg (N=8) for one month, before going to sleep. Patients were evaluated before and after treatment by IIEF test and Duplex Sonography with PG1. RESULTS. In Sildenafil group mean cavernosal PSV resulted 20.8±3.8 before and 33.1±8.5 after treatment (p=0.002). In Placebo group mean cavernosal PSV was 18.9±3.5 before and 22.2±8.8 after treatment (p=N.S.). In Sildenafil group mean IIEF value was 12±7.6 before and 19.1±8.6 after treatment (p=N.S.). In Placebo group mean IIEF value was 6±3.9 before and 10.4±11.1 after treatment (p=N.S.). CONCLUSION. A daily administration of a low dose of Sildenafil significantly increases impaired penile circulation in vasculogenic impotent patients. Our data support a real therapeutic role of oral Sildenafil in impotent males.

P5/6 – 113

PREVALENCE OF ERECTILE DYSFUNCTION AND SILDENAFIL USE IN CALIFORNIA.
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The epidemiology of erectile dysfunction (ED) is a topic of medical, social and economic importance. We evaluated the prevalence of ED using a validated self-administered 5-item questionnaire (IIEF-5) and examined the use of sildenafil in community-dwelling men. From 1972-1974, 82% of adults in Rancho Bernardo, a middle-class community in southern California, enrolled in a heart disease risk factor study. In October 1998 the IIEF-5 was sent to all surviving members and a second mailing sent to non-respondents in March 1999. Degree of ED was classified by the erectile function domain score as complete (<4), severe (5-10), moderate (11-14), mild (15-18), and none (19-20). Sildenafil utilization and effectiveness was recorded. Nine hundred seventy-six men (64%) returned completed questionnaires. Mean age was 65, with 33% aged 75+. Internal consistency of the questionnaire construct was confirmed with a Cronbach's alpha coefficient of 0.96. No sexual activity was reported by 23% of all men and 48% of men aged 80+. "Low" or "very low" confidence in ability to keep an erection was reported by 37% of all men and 70% of men 80+. Twenty-five percent of sexually active men reported complete (3%), severe (10%) or moderate (12%) ED based on the erectile function domain score. Fifty-seven percent of men 75-80 reported moderate to complete ED. The prevalence of sildenafil use was 11%. A good or terrific response was reported by 59%, while 15% reported no response. Response rate was better in men <70 (78%) compared to men >70 (43%). This contemporary population-based survey confirms the strong correlation between age and prevalence of ED and dissatisfaction with sexual performance. We document the significant impact of sildenafil on ED in a community-based study during the first 10 months of availability.

P5/6 – 114

EFFECTS OF SILDENAFIL ON SEMEN QUALITY AND MALE ACCESSORY GENITAL GLAND (MAGG) FUNCTION.
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Collection of semen samples (SSs) of the highest quality is of great importance in assisted reproduction. We evaluated the influence of sildenafil on semen parameters and MAGG function in infertile men. Each of 13 oligozoospermic infertile men produced three SSs via masturbation after 3 days of abstinence. The same 13 men produced three additional SSs after administration of sildenafil (50 mg; same period of abstinence). Total sperm count (TSC), % motile sperms (%MMS), and % morphologically normal sperms (%MNS) were evaluated in all 39 samples (group A) collected without sildenafil treatment and in all 39 samples (group B) collected after sildenafil treatment. The first, second and third SS collected from each participant via each method were processed for evaluation of a-glucosidase (marker of epididymal function), fructose (marker of seminal vesicular function), and citrate (marker of prostatic secretory function; PSF), respectively. TSC, %MMS, and %MNS significantly larger (p<0.05) in group B samples than in group A samples. In contrast, differences in %MNS, fructose, and a-glucosidase between A and B were not significant (p>0.05). Differences in prostatic secretions and TSC between groups A and B may be due to the greater sexual stimulation during ejaculation in group B that promoted PSF and increased the loading of vas deferens. The increase in PSF after sildenafil treatment explains the enhanced sperm motility.

P5/6 – 115

EFFECTS OF SILDENAFIL ON SLEEP-RELATED ERECTIONS IN NORMAL MEN.
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Sildenafil is an efficacious treatment for psychogenic and mild organic penile erectile dysfunction (ED). We studied the effects of Sildenafil on sleep-related erections in men not affected by erectile dysfunction. Twenty adult healthy men (mean age: 36.4 ± 10.7 years) underwent nocturnal penile tumescence and rigidity monitoring (NPTRM) on 3 consecutive nights, being the first an adaptation night. All subjects received 50 mg of Sildenafil orally 1 hour before starting NPTRM: the drug was administered to 11 subjects the second night and the other 9 subjects the third night. We evaluated the following parameters during all the 8 hours (8hT), during the first 4 hours (1-4hT) and the second 4 hours (5-8hT) of the test: total number of valid erections, maximum increase of tumescence, total duration of maximum increase of tumescence, total time of increase in penile circumference > 30 mm, maximum rigidity, total duration of the maximum rigidity, total duration of rigidity > 70%. Student's t-test for paired data was performed to compare: 8hT with vs without Sildenafil, 1-4hT vs 5-8hT without Sildenafil, 1-4hT vs 5-8hT with Sildenafil, 1-4hT with vs without Sildenafil, 5-8hT with vs without Sildenafil. After Sildenafil administration the 7 parameters resulted significantly higher than without Sildenafil (p<0.05). All parameters, except total number of valid erections and total duration of maximum increase of tumescence, were significantly higher in 5-8hT than in 1-4hT; only with Sildenafil administration (p<0.01). These data suggest that Sildenafil significantly improves sleep-related erections in normal men and that the efficacy seems greater after more than 5 hours from administration.
P5/6 – 116
ALFUZOSIN PHARMACOTHERAPY IN PRIMARY PREMATURE EJACULATION
Dr. Vipan Bhatia*, Department Of Urology & Andrology, Oasis Hospital, Al Ain, United Arab Emirates
Introduction: Primary premature ejaculation (PPE) continues to be a common and distressing quality of life disorder. Accepted treatment options include psychotherapy and oral pharmacotherapy with tricyclic antidepressants or selective serotonin reuptake inhibitors (SSRIs). Objective: This placebo controlled double blind study evaluated the therapeutic efficacy of Alfuzosin a uroselective alpha 1 blocker in the management of PPE. Material & Methods: The patient population comprises 43 males, mean age 29 years (24-41 years). All the patients had a stable female sexual partner with normal sexual habits and practice. The group complained of premature ejaculation which was defined as intravaginal ejaculation latency time (IELT) of less than 120 seconds during more than 50% of sexual intercourse. The mean IELT was 40 seconds. Patients with erectile dysfunction, psychiatric disorders, history of urethritis or prostatitis, concurrent significant medical disease, alcohol or substance abuse and simultaneous use of drugs affecting sexual function were excluded from the study. All patients had detailed history taking and general physical examination including digital rectal examination. Laboratory investigations included blood sugar, urine culture and sensitivity and post prostate massage urine examination were performed. All patients were informed about the study but the individual nature of medications was not disclosed. Each patient received a multivitamin capsule twice a day for one month followed by no treatment for 4 weeks and then followed by one month therapy with Alfuzosin 5 mgm twice a day. Results: The factors evaluated included: A) IELT in seconds in the last three sexual intercourses as recorded by the patient, B) sexual satisfaction-graded I, II, III (fair, good, excellent), C) onset of improvement in IELT.

P5/6 – 117
THERAPEUTIC ROLE OF SHORT INTRACAVERNOUS ALPROSTADIL IN ARTERIOGENIC IMPOTENT PATIENTS
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INTRODUCTION. Previous Duplex sonography (DS) studies provided evidences of an improvement of Peak Sistolic Velocity (PSV) in cavernosal arteries after repeated self-injections of Prostaglandin E1 for a long time (12-36 months). The aim of this study is to find out if a short term penile intracavernous therapy is able to increase PSV in cavernosal arteries in arteriogenic impotent patients.
MATERIALS AND METHODS. Twenty-one patients were evaluated by IIEF test and DS with 10 μg PGE1 before and after weekly intracavernous injection therapy with Alprostadil for four weeks. We classified subjects with cavernosal PSV >30 cm/s as group A and subjects with PSV <30 cm/s as group B. RESULTS. In group A mean cavernosal PSV values were 42.7 ±11.1 before and 42.8±11.3 after intracavernosal treatment (p=N.S.). In group B mean cavernosal PSV values were 20.4±5.2 before and 28.3±9.2 after treatment (p = 0.0008). In group A mean IIEF value was 15.1±4.6 before and 21.9±2.8 after treatment (p=0.0009). In group B mean IIEF value was 14.0±4.9 before and 19.5±5.4 after treatment (p=0.021). CONCLUSION. A short Alprostadil treatment can increase impaired penile circulation in vasculogenic impotent patients.

P5/6 – 118
COMPARATIVE STUDY OF EFFECT OF DOTHIEPIN, TRAZADONE AND FLUOXETINE IN TREATMENT OF IMPOTENCE SECONDARY TO DEPRESSION
Bhupendra Sharma* And C M. Sharma, Department of Neurology, S.M.S. Medical College and Hospital, Jaipur, India.
75 male patients age 25-50 years complaining of loss of libido for more than 8 months duration were studied. Patients with organic causes of impotence were not included in the study. Beck depressive inventory and especially designed questionnaire were used for assessment of depression before and after the treatment. Patients were divided in three groups. In group I patients received dothiepin 75 mg as a single dose at bedtime orally for four months. In group II patients received Fluoxetine 20 mg as a single dose orally for four months. In group III patients received Trazadone 100 mg as a single dose orally for four months. In group I patients started showing improvement in sexual function within two months of therapy & at the end of four months 15(60%) patients out of 25 showed marked improvement in sexual function. In group II 11 (42%) patients out of 25 showed improvement in sexual function after four months of treatment. In group III 12 (48%) patients out of 25 showed improvement in sexual function after four months of treatment. There were no significant side effects seen with these drugs. In conclusion dothiepin is more effective, and having quicker action in the treatment of impotence secondary to depression when compared with Fluoxetine & Trazadone.

P5/6 – 119
COMPARISON OF VASOACTIVE NEUROPEPTIDES IN THE HUMAN PENIS AND CLITORIS
A Jungwirth, C Hauser-Kronberger*, K Fink*, N Schmeller*, Salzburg General Hospital, Salzburg, Austria
Introduction: A large number of biologically active peptides have been identified in the nervous structures of the urogenital system. They are suggested to be involved in the local control of blood flow and smooth muscle relaxation during sexual excitation. Material and Methods: Specimens from whole human penises (n=4) obtained from gender reassignment and clitoris from patients undergoing clitoridectomy because of an adenogenital syndrome (n=1) and for oncological reasons (n=3) were analyzed. Using immunohistochemical methods, vasoactive intestinal polypeptide (VIP), peptide histidine methionine PTHM), pituitary adenylate cyclase activating peptide (PACAP), helospectin (HS) and neuropeptide tyrosine (NPY) were detected. Results: The potential vasodilators VIP, PTHM, PACAP and the vasoconstrictor NPY were distributed mainly at the border of the tunica media and adventitia of arteries. Moderate single nerve fibers containing VIP, PTHM, HS and NPY were distributed between the smooth muscle cells of the trabecular tissue. The distribution pattern of the general and peptidergic innervations in the human penis is similar to that of the clitoris, although the penis has more extensive innervation. The overall distribution of nerve fibers showing immunoreactivity for vasoactive neuropeptides was highest in the penile cavernous body followed by the spongiosus body and the cavernous body of the clitoris. Conclusion: The distribution of the peptide immunoreactivities demonstrated in the cavernous and spongiosus bodies of the human penis and the cavernous body of the clitoris indicate their role in regulation of local blood flow and may be promising substances in the onset of therapy of E.D.
P5/6 – 120

YOHIMBINE TREATMENT OF ORGANIC ERECTILE DYSFUNCTION (ED) IN A DOSE ESCALATION TRIAL.

AT Guin*, RF Sparh*, JL Jacobson, FT Murray, ME Geisser, Lahey Clinic Center For Sexual Function, Peabody, MA.

The use of yohimbine as a treatment for ED has been debated for years. Moderate success in psychological impotence is accepted by many but the effect in organic ED has been felt to be minimal. The authors noted a lack of efficacy of yohimbine in current smokers. It was felt that a reevaluation of the drug in non-smokers who have an etiology for their ED was warranted. MEIT IODS: 18 men, mean age 60.2 years (range 34–69), were recruited during initial evaluation for ED. Organic ED was confirmed by the Florida Sexual Health Questionnaire (FSHQ) and by abnormal nocturnal erections with RigiScan evaluation. Vital signs, nocturnal erections, questionnaires and hormone levels (testosterone and DHEA-Sulfate) were monitored after one month of 5.4 mg TID and one month of 10.8 mg TID of oral yohimbine. RESULTS: 9 of the 18 men (50%) improved their erectile function. Success was defined as completion of intercourse in >75% of attempts, verified by home diaries and the FSHQ (less difficulty in obtaining, p<.03, and maintaining, p<.01, erections for vaginal intercourse). Age was not a factor. No change in BP or pulse was noted. Cortisol levels were higher in the responders (p<.03). RigiScan penile activity was improved in the responders: tip rigidity (p<.001), base tumescence (p<.009) and a trend for base rigidity (p<.065). Free testosterone and DHEA-S levels were normal. No change was noted with yohimbine treatment. Baseline free testosterone, however, and baseline RigiScan measurements were higher in the responders vs the non-responders, although not significantly. CONCLUSION: Yohimbine was effective in a selective group of non-smoking men with organic ED, and perhaps those with less severe ED. It deserves a place in our therapeutic armamentarium.

P5/6 – 121

THE EFFECT OF NATURAL OIL AS A SOURCE OF GAMMA LINOLENIC ACID ON ERECTILE DYSFUNCTION IN PATIENTS OF DIABETES MELLITUS

C.M. Sharma* And. Bhupendra Sharma. Department of Neurology, S.M.S. Medical College and Hospital, Jaipur, India.

Fifty patients of diabetes mellitus with erectile dysfunction were analyzed by a specially designed questionnaire. A double blind placebo-controlled study to assess the effect of dietary supplement of natural oil (evening Primosa oil) as source of Gamma Linolenic Acid on the erectile dysfunction. Patients were divided in two groups. In group I twenty five patients received 1000 mg of evening primosa oil (Primosa) in BID doses and remaining twenty five patients received placebo capsules for six months. All patients were assessed at the beginning and end of the study by history, clinical examination, and interview with partners. When compared with placebo group, patients on evening Primosa oil showed statistically significant improvement in sexual function. In group I nine patients (36%) showed improvement in erectile functions as compared to group II three patients (12%) showed improvement in erectile function. There was no side effect noted in the patients receiving primosa oil. We conclude that Gamma Linolenic Acid therapy has useful role in the treatment of erectile dysfunction in patient of diabetes mellitus.

P5/6 – 122

INTRANASAL VASOACTIVE AGENTS INDUCE CYTOTOXICITY IN CULTURED HUMAN PENILE CAVERNOSAL SMOOTH MUSCLE. Manoj Monga, Valeria Pagon*, Mahadevan Rajasekaran, UCSD Medical Center, San Diego, CA

Intracavernosal pharmacotherapy with vasoactive agents for male erectile dysfunction has been associated with long-term complications such as reduction in penile smooth muscle content and fibrosis. We evaluated the direct in vitro cytotoxicity of vasoactive agents (papaverine, phenolamine and prostaglandin E1) to human penile cavernosal smooth muscle cells. Human penile cavernosal tissue explants (1-2 mm² size) were obtained with proper IRB approval from patients undergoing penile prosthesis implantation. Primary culture was initiated in DMEM-10% FBS media and monolayer cavernosal cells were grown in 96 well tissue culture dishes. At 60-80% confluency, cells were labeled overnight with 32Na, CrO4 (1.5 μCi) and then incubated with therapeutic concentrations of papaverine (6 mg/cc), phenolamine (0.5 mg/cc) and PGE1 (4.5 μg/cc) alone as well as in triple combination for 30 minutes at 37°C. At the end of incubation, an aliquot of supernatant was collected in scintillation vials. The release of cell free chromium in supernatants was determined in a liquid scintillation counter and the results were expressed as percent cytotoxicity.

Papaverine induced a significant increase in chromium release from the cavernosal cells. Up to 80% cytotoxicity was observed with papaverine, where as the triple combination resulted in 60% of cell damage. Incubation with Phenolamine or PGE1 did not produce any cytotoxicity at the employed therapeutic concentrations.

Papaverine induced cytotoxicity to cavernosal smooth muscle cells may contribute to fibrosis and loss of smooth muscle content associated with the intracavernous pharmacotherapy. Quantitative evaluation of in vitro cytotoxicity in human cavernosal smooth muscle cell culture may be important in the development of new intracavernosal agents.
**P5/6 - 124**

**CHOLINERGIC STIMULATION OF PENILE ERECTION IN MICE**  
Arthur L. Burnett, Alex G. Chang*, Sena F. Sezen*, Julie K. Crone*

The influence of cholinergic neurotransmission in the mediation of penile erection operating by stimulating the release of vasodilatory substances from the vascular and sinusoidal endothelium in the penis has been proposed. Nitric oxide, also known as endothelium derived relaxing factor (EDRF), is believed to be a major substance synthesized and released under the action of endothelial nitric oxide synthase (eNOS). To investigate this possibility in wild-type mice as a prelude to evaluating cholinergic effects in transgenic mice lacking the eNOS gene, physiologic erection studies were carried out. Electrical stimulation of the cavernous nerve in the mouse with parameters of 16 Hz, 5 milliseconds, 1 to 6 volts yielded intracavernosal pressure (ICP) recordings at levels directly proportionate to voltage applied. Intracavernosal administration of the cholinergic agonist was evaluated for eroticogenic effects, with determination of a 0.003 μg dosage that did not produce adverse systemic effects. Quantifiable ICP recordings were obtained using a combination of submaximal electrical stimulation (16 Hz, 5 ms, 1 volt) of the cavernous nerve and intracavernosal carbachol administration (0.003 μg). A relatively brief time interval of 2.5 minutes from carbachol administration to electrical stimulation was determined since time intervals greater than this duration failed to show distinctive carbachol-related increases in ICP recordings over electrical stimulation alone (suggesting intrapenile wash-out effects of the drug occur with prolonged time intervals). Using combined electrophysiologic and pharmacologic stimulation, ICP recordings were raised significantly from that produced by electrical stimulation alone (from 35.3 ± 2.7 to 48.1 ± 5.5 mm Hg, n = 8, p < 0.05). These results, which establish parameters for evaluating cholinergic stimulation of erectile function in vivo in the mouse affirm that cholinergic neurotransmission mediates murine penile erection.

**P5/6 - 126**

**CIALIS® (IC351): EFFECTIVE AND WELL-TOLERATED TREATMENT FOR ED**  
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†Univ W Ontario, London, Ontario; *Eli Lilly, Scarborough, Ontario, Canada; †ICOS, Bothell, Indianapolis, USA.

**INTRODUCTION:** CIALIS® (IC351) is a potent and selective inhibitor of PDE type 5. This multifunction, double-blind, placebo (PBO)-controlled, parallel study evaluated the efficacy and safety of oral Cialis in men with mild-to-severe erectile dysfunction (ED).

**METHODS:** After a 4-wk treatment-free run-in period, during which baseline (BL) International Index of Erectile Function (IIEF) scores and Sexual Encounter Profile (SEP) diary data were obtained, 212 men received PBO or a research formulation of Cialis 2.5 mg for 8 wks. Doses were taken as needed prior to sexual activity (max. 1 dose/24 h). Endpoints (EPs) included a BL in IIEF domains and SEP diary data, as well as responses to a global assessment question (GAQ).

**RESULTS:** Compared with PBO, Cialis doses (5–25 mg) significantly improved patient scores for IIEF Erectile Function, Organic Function, and Overall Satisfaction domains, and SEP scores. In addition, Cialis-treated patients (2–25 mg) completing the GAQ had a greater % of positive responses than PBO-treated patients (57–88% vs 28%, respectively). The most common treatment-related AE's (all Cialis >5%), headache and dyspepsia, were generally mild to moderate and appeared to abate over time.

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<th>PBO (n=41)</th>
<th>Cialis (n=42)</th>
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All values are mean ± standard deviation

**CONCLUSIONS:** In this study, Cialis (5–25 mg) was superior to PBO and was well-tolerated when administered on demand to men with mild-to-severe ED.

**P5/6 - 127**

**EFFECTS OF DIOXIN ON PENILE ERECTION IN THE RABBIT**  
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Introduction and Objectives: Recently, dioxin (TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin) has received much attention worldwide as an endocrine disruptor. Garbage incinerators, exhaust from leaded gasoline and the pulp industries generate TCDD, one of the most potent environmental pollutants. Decreases in spermato genesis and the ability to conceive and carry a pregnancy to term are the most sensitive signs of reproductive toxicity by TCDD in the mammal but no report of its effect on erectile function exists. We performed this study to investigate the effect of dioxin on the erectile function. Methods: In four groups of New Zealand white rabbits, Group I: control, II: 4 weeks after 1μg/kg of TCDD (i.p.), III: 8 weeks after 1 μg/kg of TCDD, cavernosal tissue strips were harvested for pharmacological study in the organ bath. Isometric tension was established with phenylephrine (10-6M). Contractions were studied with norepinephrine and relaxations were studied with acetylcholine and sodium nitroprusside. In a separate experiment, histological examination was performed in the animals of corresponding groups. Results: Compared to Group I, the contractions and relaxations of cavernosal strips were significantly suppressed in Group II and III (p<0.01). In Group III, even higher concentration (10-3M) of acetylcholine did not relax the precontracted strips. Histologically, thickening of tunica albuginea with markedly increased collagen fibers, subterminal deposition of fat, decreased sinusoidal space by severe cavernosal smooth muscle hyperplasia and cavernosal fibrosis were identified in all TCDD-treated animals. Conclusions: These results suggest that dioxin leads to erectile dysfunction via penile histologic changes in rabbit.
P5/6 – 128
BIPHENOL A INHIBITS PENILE ERECTION VIA ALTERATION OF PENILE HISTOLOGY IN THE RABBIT
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Introduction and Objectives: Xenoestrogens have been shown to affect reproduction in wild life and may have adverse effects on humans because of their ubiquitous presence in the environment, resistance to degradation, and potential for accumulation in fat tissues. Despite extensive researches in the toxicity of bisphenol A (BPA), no report of its effect on erectile function exists. We performed this study to investigate the effect of BPA on erectile function. Methods: In four groups of New Zealand white rabbits, Group I: control(n=7), II: 8 weeks after 1 mg of BPA (intracavernosal, n=7), III: 8 weeks after 1 ng of BPA (intracavernosal, n=7), IV: 8 weeks after 1pg/kg of BPA (intracavernosal, n=7), cavernosal tissue strips were harvested for pharmacochemical study in the organ bath. Isometric tension was established with phenylephrine (10^5M). Contraction were studied with norepinephrine and relaxations were studied with acetylcholine, L-arginine and sodium nitroprusside. In separate experiment, histological examination was performed in the animals of corresponding groups (Gr. I: n=7, Gr. II: n=7, Gr. III: n=7, Gr. IV: n=7). Results: In all BPA treated groups, the contractions and relaxations of cavernosal strips were significantly suppressed compared with Group I (p<0.01) in a dose-dependent manner. Histologically, thickening of tunica albuginea and deposition of fat, decreased sinusoidal space by severe cavernosal smooth muscle hyperplasia and cavernosal fibrosis were identified in all BPA-treated animals in a dose-dependent manner. Conclusions: These results suggest that BPA leads erectile dysfunction via alteration of penile histology even at a dose of pg. On following studies, the effects of BPA on human penis should be elucidated.

P5/6 – 129
TRANSPENILE DELIVERY OF MICROVASCULOKINETIC DRUGS (TRAPS) IN THE MEDICAL TREATMENT OF ERECTILE DYSFUNCTIONS FOR PENILE REHABILITATION.
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Transpenile Barrier Systems (TRAPS) as passive Transderm al Barrier(TDB) or active Transp eidermal Barrier(TEB) are emerging methods to delivery specific vasoactive drugs for erectile tissue dysfunctions and rehabilitation. Compounds with microvascularkinetic activity,complexed with phosphatidylcholine are tested in clinical trials for vasogenic impotence to restore the metabolic balance of the erectile tissue. A preliminary phase III study is realized using the phosphatidylcholine/troxerutin complex (3%) daily applied for 3 months on the penile skin in a selected group of 24 peer-patient (aged between 20 and 55), affected by a severe (micro)vascular disease with a long standing erectile dysfunction. Number of spontaneous daily erections, duration and rigidity, IOPVC (Optic Video Capillaroscopy), LDF (Laser Doppler Flowmetry),thermic and NO-microsensors measurements are collected at TD0-T1-T2-T3. The global primary outcome shows at Day 90 the positive response of Troxerutin(Tr) versus Placebo(P):Tr19/24(79.2%);P1/24(4.2%);no difference is found in 4/24(16.3%). In the secondary outcome evaluation the mean duration of spontaneous daily erections improves gradually from 5.4 to 8.0 in Tr and it decreases from 6.0 to 1.6 in P. The LDF recordings show a clear difference in the A score between P and Tr: P70=6.99,P30=7.77,P60=5.81, P90=8.41/Tr70=48.76,Tr30=63.12,Tr60=74.72,Tr90=81.77(p<0.001 TO,T30,T60,T90). This is the first clinical trial with a TRAPS system designed to improve the microvasculokinetic activity of the erectile tissue in which microtechnologies dedicated to microcirculation and metabolic parameters are used in vivo in clinical studies for the rehabilitation of the erectile tissue.

P5/6 – 130
IS ABNORMAL FSH PRECLUDING PATIENTS FROM DOING TESTIS BIOPSY OR TESTE?
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Objective: To determine predictive value of abnormal FSH as an index of spermatogenic failure in comparison to results of testis biopsy. Design: Prospective study. Setting: University infertility center. Materials and methods: Sixty infertile males with azoospermia underwent testicular biopsy and/or testicular sperm extraction (TESE). An hormonal study to determine serum FSH, LH and Testosterone level was done for all of them. Those patients who had spermatooza or spermaticid in their specimens underwent intracytoplasmic sperm injection (ICSI) cycles. Results: Of the sixty patients that were studied,fourty-five had abnormal FSH levels and the hormonal profile in the other 15 patients were normal. Testis biopsy and/or TES in the former group showed no spermatooza or spermaticid in six of them.In the other 15 patients only spermaticid was seen in their specimens, but twenty-four patients had either motile or mature immotile sperm in their testis tissues. Therefore microinjection was tried for them. Conclusion: Before the presentation of microinjection(ICS1 for the treatment of severe male factor infertility with the usage of sperm extracted from testis,infertile men with azoospermia and an elevated level of FSH were excluded from further treatment trials. This study showed that abnormal FSH should not preclude patients from further diagnostic and treatment trials because even in these patients can be found good candidates for ICSI programs.

P5/6 – 131
TREATMENT OF IDIOPATHIC OLIGOZOOSPERMIA WITH rh-FSH
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Introduction. FSH is required for normal spermatogenesis but the treatment of male infertility is still matter of debate. In this study we examined the role of rhFSH therapy in male idiopathic infertility. Patients and methods. A group of 30 oligozoospermic subjects (sperm < 10 mil/ml) was selected on the basis of clinical parameters predictive for response to FSH therapy as demonstrated previously: normal FSH and inhibit Β (INHB) plasma levels and a testicular cytokine showing moderate hyperospermogenaisis without maturative disturbances. Patients were randomly divided in 3 groups: in group A 10 patients were treated with rhFSH (Puregon, Organon) at the dose of 50 IU on alternate day for 3 months; in group B 10 patients were treated with rhFSH at the dose of 100 IU for the same period; in group C 10 subjects were kept without any treatment. FSH, LH, T and INHB plasma concentrations were measured weekly for the first month of therapy and then monthly; semen analysis was performed monthly while fine needle aspiration cytology (FNAC) was performed before and after therapy. Results. In group A we did not observe any variation of FSH and INHB plasma levels during therapy, while in group B these hormones increased after 2, 3 and 4 weeks of therapy with respect to pre-treatment levels, group A and group C (p<0.001) as well as sperm concentration. In group B we detected an increase of spermatogonia and spermatocyte compared to pre-treatment values as evaluated by FNAC (p<0.05). Conclusion. This study shows that in idiopathic oligozoospermia, in presence of isolated hyperospermogenesis, normal FSH and INHB plasma levels, rhFSH at the dose of 100 IU on alternate day for at least 3 months stimulates spermatogenesis increasing sperm number.
**Abstracts – Poster Session 5/6**

**P5/6 - 132**

**DOES CLOMIPHENE CITRATE IMPROVE FERTILIZING POTENTIAL OF SPERMATOZOA?**


Clomiphene Citrate (CC) is a drug commonly used empirically to improve spermatozoa. The success is variable due to various factors. Objective: To evaluate the benefits of two different drugs intake schedule on the fertilizing potential of spermatozoa in idiopathic subfertile males. Methodology: 20 idiopathic subfertile men (25-40 years) were recruited for post-treatment semen analysis (count, motility, morphology) and sperm functional test for hyposmotic swelling (HOS) test, sperm mitochondrion index (SMAI), nuclear decondensation (NCD) and gelatin test for sperm acrosin; gondatropin and serum testosterone were also measured. CC 25 mg daily (N = 9) and CC 25 mg alternate day (N = 11) x 3 months were allocated in a randomised manner. The post treatment parameters were evaluated as above after completion of therapy. Results: All the subjects had primary infertility and had an average age of 32 years. Post treatment improvement in count and motility 82% and 55% respectively, was seen in the alternate day group, while as only motility showed an improvement (63%) in the daily dose group. The improvement was in the subfertile range. Functional parameters HOS and SMAI improved in alternate day and in daily dose only HOS showed improvement. All were in the subfertile range. Post treatment increase of 25% from basal values of FSH and testosterone were seen. Conclusion: Our study showed a 50% improvement in fertilizing ability of spermatozoa in the subfertile range. The alternate day group fared better. Non responders could due to local intrinsic genetic factors causing hypospermatozoa.

**P5/6 - 133**

**INHIBIN B IS A HORMONAL MARKER OF THE ADVERSE EFFECT OF CRYPTOCHIDISM ON SPERMATOGENESIS**

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OBJECTIVES: To investigate retrospectively the incidence of cryptorchidism and the impact of cryptorchidism on spermatozoa, testicular volume, inhibit β, FSH, LH, testosterone and SHBG concentrations. METHODS: A total of 267 subfertile patients attending the Andrology outpatient clinic with a history of undescended testes were included. Unilateral cryptorchidism was present in 162 patients; bilateral in 105 patients. From the total group of 2613 infertile men, 520 noncryptorchid control patients were randomly selected. The cryptorchid and controls patients were matched according to sperm concentration. RESULTS: The incidence of cryptorchidism was 10.2%. Inhibit β concentrations (mean±SD) in normozoospermic (>20x10⁶/mL)men were not significantly different between cryptorchid (153.5±/60.1) and controls (178.6±66.1 ng/L). In oligozoospermic (<20x10⁶/mL) cryptorchid men, inhibit β concentrations were significantly lower (P<0.05) than in controls (103.3±75.3 and 136.3±76.1 ng/L, respectively). FSH concentrations were significantly (P<0.01) higher (9.3±10.0 and 6.8±7.1 IU/L, respectively). No significant differences were found between testosterone, LH and SHBG concentrations of both cryptorchid and noncryptorchid groups and neither. The volumes of both right and left testis were significantly smaller (P<0.01) in oligozoospermic cryptorchid men than in controls. CONCLUSIONS: This study shows that cryptorchidism has an adverse effect on spermatogenesis and Sertoli cell function, reflected by low serum concentrations inhibit β.

**P5/6 - 134**

**CORRELATION BETWEEN SEX HORMONES AND PARAMETERS OF SPERMOMGRAM IN MALE ACCESSORY GLAND INFECTION.**

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Male accessory gland infection (MAGI) may interfere with the reproductive potential and can result in glands secretory dysfunction. Endocrinological disorders play an important role in pathogenesis of infertility. This study correlates the level of sex hormones in serum and seminal fluid with different parameters of semen. 60 patients were investigated using WHO simplified management of infertile couple. The control group included 67 healthy fertile volunteers. Semen and sex hormones (FSH, LH, Prolactin, Testosterone, Estradiol, Progesterone, Cortisol) were analysed according the WHO guidelines. Serum and seminal fluid hormones were measured using RIA and EIA. In infertile patients with MAGI statistically significant low seminal volume, sperm density and motility were found (p<0.001). Concentrations of Fuctose, Acid Phosphatase, Citric acid, Zinc and ATP were low in comparison with controls. Regression analysis performed between sex hormones and parameters of semen revealed, that pH of semen correlate highly with concentration of serum Prolactin(r=0.699, p<0.01). % of motile spermatozoa with rapid linear progression with level of Testosterone (r=0.732, p=0.01) and % of normal forms with Cortisol(r=0.58, p<0.05).

**P5/6 - 135**

**THE FUNCTION OF MEN REPRODUCTIVE SYSTEM, WHICH WERE PREPARED FOR PROGRAM EXTRA - CORPORAL FERTILIZATION.**


In a joint effort with the City Human Reproduction Center we examined 320 couples of those received treatment under extra corporal fertilization program (ECF), out of which those suffering female infertility made up 55%, male infertility - 35% and a mixed form - 10%. The examined group of the males covered by the extra-corporal fertilization program included 150 patients with the ages ranging from 27 to 40 years old. In anamnesis only 30% of the males examined had conserved fertility. Quite a high percentage (80%) of those had suffered childrens infections (chicken pox, mumps, scarlet fever, German measles, measles); 12% of the males had allergic reactions, 20% had excessive weight. Indicators of the sperm test were a key parameter for assessing a functional condition of the males' reproductive system. Analysis of the sperm test parameters of the examined patients revealed that volume of the sperm was below the physiological norm for 80% of those examined. Only 10% of the patients had a number of spermatozoa which was within a physiological norm. Analysis of spermatozoa agility revealed that 45% of the patients had a significantly lower indicator of this parameter. The infection indicator in the sperm test showed an elevated number of leukocytes in it (among 11% of the examined program patients). In connection with the revealed dysfunctions in the sperm test parameters among the males covered by the program they were subjected to the endocrine examination. It was established that in terms of production of the key male hormone - testosterone, the program patients differed from practically healthy males.
P5/6 – 136
ANALYSES OF SPERM QUALITY IN LARGE POPULATIONS OVER DECADES BY "DATAMINING" AND "DATWAREHOUSING" (WINSPERMLE)
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Objective The analysis of the variations of semen parameters in large populations over long periods demands multicenter acquisition of data and new methods, such as "Dataminning (DMG)" within a "Data Warehouse (DWH)". DWH are setup on operational databases which are not available for specialities up to now. Our objective is the presentation of Winsperm2001 (WSP), a specialized workflow database for standardized acquisition and evaluation of data of infertile couples as a prerequisite of evidence medicine. Furthermore, WSP was applied for analysis of sperm qualities in our region over decades. Methods Based on a standardized evaluation program of infertile couples an electronic database has been developed. WSP is using the relational database management system Access8.0® for Windows 9x/NT® with an integrated workflow management system for networks. All data are stored in 4 backends, 105 tables and 1000 fields. Results WSP is used at 5 university centers in Germany and another 3 centers are going to implement it this year. Up to December of 2000 the DWH contains 26.643 couples and 180.000 clinical record sets. An analysis of 3432 patients born between 1952 and 1971 in the region of Leipzig (Germany) showed significantly lower sperm concentrations and reduced total sperm counts in men born after 1959 compared with men born before this year (52.4±1.2 vs. 70.8±2.2 Mio ml-1, 139.5±3.7 vs. 176.3±6.0 Mio; meansSEM; p<0.01; 2410 vs. 1022 patients) independently of the time of semen examination. Conclusion WSP realizes the first step toward an andrological DWH. The first evaluation of the system at our centers revealed significantly different sperm counts in dependence on the year of birth. A trial version can be obtained.

P5/6 – 138
DOES MALE SEXUAL DYSFUNCTION INFLUENCE MALE INFERTILITY?
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Progress of science and technology has proved that male sexual function and ejaculated semen are two different aspects that may influence male infertility. Erectile Dysfunction (ED) may cause indirect infertility problems, but this patient may be fertile when semen can be collected for the application of ART. Libido, ejaculation, orgasmic disorders may interfere with the mechanism of ejaculation and produce inferior composition of semen, causing male infertility. Proper ejaculation is of paramount important to harvest good semen composition. A study of 86 husbands married between 1 year and over suffering premature ejaculation (PE) were performed and evaluated for their fertility status and semen quality. 71 husbands have fathered their children and 4 more others conceived their wives. 11(12.9%) husbands did not succeed to conceive their wives. Semen analysis performed on 8 of these husbands show bad results (oligoasthenoterato-zoo spermia). The other 3 husbands showed normal semen parameters (normozoospermia). The fertility potency of married husbands with ED did not differ significantly with normal husbands (WHO report, 1972; male infertility 11-12%). Primary oral (with Clomipramine), topical (anesthetics, SS-cream)and surgical (dorsal nerve neurotomy) treatments for PE would not be recommended for the management in male infertility, because it may worsened semen quality Treatment for other sexual dysfunctions with infertility, such as problems with libido (protodioscine), ED (PGE-1, phospho-diesterase inhibitor), an., retrograde (Le' plasir sec), and sham-ejaculation will be further discussed. Keyword: Male sexual dysfunction, semen quality, male infertility.

P5/6 – 137
MALE INFERTILITY RISK FACTORS IN A FRENCH MILITARY POPULATION (RESULTS OF A CASE-CONTROL STUDY)
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Objective: To investigate infertility risk factors. Design: Case-control study. Setting: The military population of the French town of Brest. Participants: Sixty couples who had sought medical advice for infertility of more than 12 months duration (cases), were compared with 165 couples who had had a child (controls). All the men in these couples had been employed by the military. Main outcome measures: Medical infertility risk factors, occupational and environmental exposures. Results: We obtained age-adjusted odds ratios of 7.4 [95% CI:1.4-39.5] for testis surgery, and 13.0 for varicocele [95% CI:1.4-120.3] in men. In logistic regression, the age-adjusted odds ratio for men having worked in a nuclear submarine was found to be 2.0 (1.0 to 3.7), and that for heat exposure was 4.5 (1.9 to 10.6). Conclusions: In addition to well known medical factors, some types of occupational exposure (heat or working in a nuclear submarine) may also be risk factors for infertility.

P5/6 – 139
TEAM MANAGEMENT OF MALE INFERTILITY FROM SPINAL CORD INJURY.
Spinal cord injury (SCI) usually causes severe impairment of fertility. In addition to inability to ejaculate, sperm may be damaged by reactive oxygen species from leukocytes and spermatogenesis may be defective. Assisted reproductive technology (ART), particularly intracytoplasmic sperm injection (ICSI), has improved the prognosis for fertility. Teamwork and close cooperation between Rehabilitation and ART services can lead to optimal management and opportunities for clinical research. We have found normal semen can be collected by electroejaculation (EE) in about 50% in the acute stage of SCI after spinal shock resolves (Lancet 343, 1072,1994). This semen can be cryopreserved. After about two weeks sperm production deteriorates. In chronic SCI, provided there is no lower motor neurone lesion, semen can usually be obtained by vibroejaculation (VE) or EE, but the quality is variable. Often there is necrospermia: low percentage motility and live sperm which improve with repeated daily ejaculation (Fertil Steril 74:221,2000). We offer sperm collection and storage in the acute phase for selected patients with complete SCI desiring children. For chronic SCI, the possibility that VE can be performed safely at home is evaluated. If semen quality improves sufficiently with regular VE, the couple are taught to self inseminate. If VE is unsuccessful, produces inadequate sperm or causes autonomic dysreflexia, EE is used daily over 2-4 days. Cryopreserved sperm with adequate post thaw motility can be used by artificial insemination (AI). ICSI is reserved for patients with semen too poor for AI, if AI is unsuccessful or when sperm are collected surgically from the testes or genital tract. Minimising intervention provides cost effective management.
Abstracts – Poster Session 5/6

P5/6 - 140
TRANSRECTAL ULTRASOUND GUIDED SEMINAL VESICULOGRAPHY IN EVALUATION OF AZOSPERMIC PATIENT
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Abstract: Transrectal guided seminal vesiculography was done for 15 azospermic patients. Twenty-two vesiculograms were performed. All patients had patent ejaculatory ducts, evident by spilling of the injected diluted non-ionic contrast media in the urinary bladder. Eight patients (36.5%) proved to have patent vas deferens traced down to the epididymis. Bilateral obstructed vas deferens were found in the remaining seven patients (63.5%). Three (13.5%) at the pelvi-inguinal region and 10 (45.5%) obstructed vas deferens were found at scrotal level. Ampullary obstruction of vas deferens was detected in one patient (4.5%). Five patients had previous operative interference. History of herniorrhaphy in three patients, scrotal surgery in two patients and pelvic urologic surgery was elicited in another patient. Two patients had a past history of bilateral epididymitis. Mild transient complications were encountered, hemospermia in one patient and perineal discomfort in another patient. We conclude that transrectal guided seminal vesiculography is a simple, safe and efficient procedure in evaluation of azospermic patient and in definition of the level of obstruction of the male genital tract.

P5/6 - 141
MEASURING MALE FERTILITY. Epidemiologic considerations.
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In demographers terminology fertility is a measure of liveborns and the male part will be represented by live borns to a given father. In populations practising birth control and seeking infertility treatment if needed this measure will be a poor correlate of fecundity, the capacity to reproduce. In epidemiology quantitative measures of fecundity have been based upon time to pregnancy (TTP) studies, or use of some type of semen biomarkers. These measures are based upon correlates of fecundity but are not measuring fecundity directly.

Time to pregnancy is the most direct measure. It is a couple characteristic which require some kind of pregnancy planning to be recorded. TTP studies could be based upon sampling conditional on a pregnancy or random sampling from the population. Strength and limitation in both approaches will be stated.

Studies on semen parameters are independent on pregnancy planning but they require consent and non-responders may be biased by self-selection related to the fecundity expectation. Relatively little is known about how well semen characteristics correlate with male fecundity and no scoring system is available for putting all information into a single index. Experience with the use of semen data will be presented.

P5/6 - 142
TIME TO PREGNANCY AND SEMEN PARAMETERS: A CROSS-SECTIONAL STUDY AMONG FERTILE COUPLES FROM FOUR EUROPEAN CITIES.

Methods: In a sample of 942 couples from 4 European towns who conceived their last pregnancy without any medical intervention, we investigated the relations between the semen quality and the waiting time to pregnancy (TTP), that is the number of months of unprotected sexual intercourse before conception. Three to nine months after conception, the pregnant women answered a questionnaire, and their male partners provided a semen sample. Sperm motility and concentration were estimated in each city. The proportion of morphologically normal spermatozoa and the multiple anomalies index (MAI, ratio of the total number of anomalies to the number of abnormal spermatozoa), were centrally estimated in an laboratory. We estimated rate ratios (RR) for the occurrence of a pregnancy by a discrete survival model, adjusted for sexual activity and female factors affecting fecundity. Results: Increasing sperm concentration influenced TTP up to 55 million/mL. We observed no association between TTP and proportion of motile sperm. The proportion of normal spermatozoa had an influence on the probability of pregnancy up till 19% (David's criteria). An increase of 0.5 in MAI was associated with an increased RR for the occurrence of a pregnancy of 0.68 (95% CI [0.54-0.85]). Conclusion: Both the proportion of normal sperm and the sperm concentration had an influence on the TTP up to relatively high values. MAI had an influence on TTP on its whole range of variation. These results obtained among fertile couples highlight the prognostic interest of semen morphology parameters to evaluate male fecundity.

P5/6 - 143
VASEOEPIDIDYMOLOGY AND VASOVASOSTOMY ARE ASSOCIATED WITH RETENTION OF SPERM CYTOSPLASMIC DROPLETS
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INTRODUCTION AND OBJECTIVES It has been shown that spermatozoa with retained cytoplasmic droplets have defective function and elaborate excessive levels of reactive oxygen species (ROS). There is some evidence to suggest that vasectomy reversal is associated with excessive ROS production in semen but the exact mechanism remains unknown. As such, we sought to determine whether vasovasostomy (VV) or vasoepididymostomy (VE) is associated with retention of sperm cytoplasmic droplets. METHODS We retrospectively reviewed patients who underwent microsurgical VV (n=15) or VE (n=16, end-to-side to head of epididymis) from 1998 to 2000. A cohort of fertile men presenting for vasectomy were used as controls (n=20). The percentage of spermatozoa with cytoplasmic droplets was detected by microscopic examination of Papanicolaou smears with a minimum of 200 spermatozoa examined per slide. RESULTS The percentage of cytoplasmic droplets in the VE and VV groups combined was significantly higher than controls (7.39 +/-0.92 vs 3.23 +/-0.43, P=0.008). There was no significant difference in cytoplasmic droplets between the VV and VE groups (7.40 +/-1.46 vs 7.38 +/-1.19). Between the combined VEVV groups and controls there was a significant difference in sperm concentration (25.6 +/-4.6 vs 43.6 +/-6.2, P<0.0001), motility (34.4 +/-4.4 vs 61.4 +/-3.2, P<0.0001) and morphology (39.6 +/-3.0 vs 56.5 +/-2.9, P<0.0002). CONCLUSIONS These data suggest that reversal of epididymal obstruction (VV or VE) is associated with impaired disposal of cytoplasmic droplets. Furthermore, the body and tail of the epididymis may not be a major source of residual sperm obstruction.
P5/6 – 144

4-D ENDORECTAL ULTRASOUND IN DIAGNOSIS OF OBSTRUCTIVE DECREASE OF FERTILITY


Introduction: Anatomical abnormalities in the ways of sperm transport can be a reason of decrease/lack of fertility. In most cases, endorectal 2-D US can visualise the level of obstruction and its reason. Yet in some cases of ejaculatory ducts obstruction or large midline cysts of the prostate, enlargement of the structures located above obstructions can be seen. The degree of enlargement of seminal vesicles and/or vas deferens, or both, often coexisting with a unilateral/bilateral partial absence or hypoplasia of one of these structures may cause difficulties in differentiation between these organs. In these rare cases, we decided to use 3-dimensional endorectal real time ultrasound called 4-D. Material and method: endorectal US of the prostate, seminal vesicles and vas deferens was done in a group of 8 patients whose 2-D endorectal US showed spectacular dilatation of seminal vesicles and vas deferens. Ultrasound was performed by Kretz Voluson 530, 730 scanners. The patients’ age ranged 24-44 years (mean 31.1). Results: in 3 cases, ejaculatory ducts obstructions were diagnosed. 5 patients had enlarged seminal vesicles with reduction of internal septations and filled with “thick” fluid. In 3 cases, compression of ejaculatory ducts by the midline cyst of the prostate was observed. In 2 cases, fibrosis and calcifications were observed in the seminal vesicles. In all the cases, 4-D endorectal US showed the cause of the obstruction and the echosstructure of dilated organs with a much better view than routine 2-D endorectal US. In conclusion, 4-D endorectal US significantly increases diagnostic possibilities, which allows to determine with greater precision the causes and the level of the occlusion of the ejaculate duct, which can help in choosing adequate treatment.

P5/6 – 146

EVALUATION OF THE EFFICACY OF TRANSRECTAL ULTRASOUND IN THE DIAGNOSIS OF EJACULATORY DUCT OBSTRUCTION IN INFERTILE MEN

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Infertility secondary to obstruction of the ejaculatory duct has previously been thought to be uncommon. However, the ability to make the diagnosis on the basis of transrectal ultrasound (TRUS) has resulted in an apparent increase in the incidence which has been reported to affect 7-14% of infertile men. Vasography, the gold standard for diagnosis of ductal obstruction, has its inherent problems and was never compared to TRUS to ascertain the accuracy and sensitivity of TRUS in the diagnosis of ejaculatory duct obstruction (EDO). We studied nineteen patients with male infertility using both TRUS and vasography and compared the results of both techniques as regards the efficacy of TRUS to diagnose EDO. The results were the same for both modalities except in one case (5.3%). In conclusion, we believe TRUS is an accurate and safe way to diagnose and help in the management of infertile males with suspected EDO.

P5/6 – 145

IS THERE A DIFFERENCE BETWEEN DIFFERENT INTERVALS AFTER VASECTOMY AND THE REPRODUCTIVE CAPACITY FROM VASECTOMIZED MENS?

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Epididymal or testicular spermatozoa have been used for Intracytoplasmic Sperm Injection (ICSI) procedure as an option for the restoration of male infertility after vasectomy in patients who does not require or have failed prior vasectomy reversal. Recently, two studies evaluated the relationship between postvasectomy period and sperm reproductive capacity after ICSI, resulting in contradictory conclusions. We evaluated the relationship between postvasectomy period and sperm reproductive capacity after ICSI. The records of 24 patients who underwent a percutaneous epididymal sperm aspiration (PESA) procedure from January 1996 to September 2000 for assisted reproductive techniques purposes were reviewed. Patient’s were classified into three groups according to the interval after vasectomy. Group I consisted of patients with an interval of 0-10 years (n = 11); Group II, 11-20 years and Group III > 20 years. No differences were seen in the mean age of the men across the three groups (44.7 ± 6.86, 44.6 ± 6.86, 44.5 ± 11.9 in Groups I, II, and III, respectively (P > 0.05). No differences were seen in the female age. 31.72 ± 5, 30.88 ± 6.27, and 34 ± 6.4 (P > 0.05). Also, no differences were seen in fertilization rates 81.9 ± 11.7, 74.11 ±16.6, and 83.5 ±11.09 (P > 0.05). Furthermore, no differences were seen in the pregnancy rate in Groups I (5/11, 45.5%); II (4/9, 44.4%); and III (1/4, 25%) (P > 0.05). Our results suggest that the interval between vasectomy and epididymal sperm aspiration with ICSI treatment has no effect on the outcome.

P5/6 – 147

ANTISPERM ANTIBODIES RECOGNISE PROSTATOSOMES

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Introduction: Antisperm antibodies (ASA) in men and women causes infertility, but the sperm antigens that are recognised by ASA are not characterised. Prostasomes, organelles secreted by human prostatic cells and expelled in seminal plasma, are immunogenic and can adhere to and fuse with the sperm. The objective of our study was to investigate if prostasomes could be antigens for ASA found in the serum of infertile patients.

Methods: We have studied the reactivity of chicken anti-prostasome antibodies and serum from 20 ASA-positive patients with human spermatozoa. The anti-prostasome antibodies were produced by immunisation of chickens with purified prostasomes. They were incubated with sperm cells and observed for agglutination. Binding of IgG and the complement component C3 were analysed by incubating the sperm cells with a chicken anti-human IgG and chicken anti-C3 antibody respectively, and analysed with flow cytometry.

Results: We found that chicken anti-prostasome antibodies caused strong agglutination of sperm cells. Approximately 90% of spermatozoa were agglutinated. All of the patients sera contained IgG antibodies against prostasomes. The results demonstrate that ASA of the IgG type in serum of infertile men and women recognised prostasomes as antigens and antibodies raised against prostasomes agglutinate human spermatozoa.
P5/6 – 148

SIMPLE METHOD TO PREDICT IVF OUTCOME
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IVF outcome is much dependent on normal sperm DNA integrity which is adversely affected by elevated levels of reactive oxygen species or diminished antioxidant capacity in semen. Sperm DNA integrity can be assessed by sperm chromatin structural assay with acridine orange, using flow cytometry. This test has high predictive value but because of high costs can not be introduced in routine infertility workup. We are introducing simple and inexpensive cytotoxic method with fluorescent dye acridine orange for straightforward visualization for the evaluation of sperm DNA integrity. This method can be introduced in routine infertility workup allowing predicting in which cases IVF can be successful and in which case antioxidant therapy would be advantageous.

P5/6 – 149

PHOSPHATIDYLINOSITOL 3-KINASE INHIBITION ENHANCES HUMAN SPERM MOTILITY IN OLIGOAESTHENOSPERMIC PATIENTS
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The male factor contributes for 40% of couple infertility. The only chance to conceive for these couples lays on assisted reproduction techniques, namely in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The high fertilization rate achieved by ICSI compared to IVF has determined an increase of ICSI application, though its absolute safety is still under debate. The recovery of a higher number of motile spermatozoa could allow oligoasthenospermic (OA) men to enter IVF rather than ICSI program. In this light, we studied the role of phosphatidylinositol 3-kinases (PI3Ks) in human sperm motility by using a PI3K inhibitor LY294002. PI3Ks are lipid and protein kinases involved in many processes of somatic cells. Motility was evaluated in swim-up selected sperm from 44 OA subjects following addition of LY. A significant increase in % forward motility was observed in all sperm samples (73.8±2.5%). LY addition directly to semen samples from 21 OA men resulted in an increase in % forward motility both in seminal plasma (35.2±4.9% vs 49.5±4.5%, p<0.001) and in the swim up recovered sperm (54.2±4.2% vs 68.9±3.7%, p<0.001). The number of sperm recovered in LY-treated samples increased (44.4±4.9%), suggesting that LY could be used to enhance sperm recovery in IVF sperm motility reduction after H2O2 addition was completely reverted by incubation with LY, indicating a protective action of LY against the detrimental effects of reactive oxygen species generated during IVF sperm selection. Our results imply that PI3K negatively regulates sperm motility and suggest LY294002 as a tool to improve recovery and motility in sperm samples prepared for IVF opening new prospective for severe OA patients.

P5/6 – 150

MICROBIOLOGICAL STUDY OF SEMEN FROM ASYMPTOMATIC INFERTILE MEN
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Objective: We have previously estimated Chlamydia trachomatis genital infection prevalence to be 24% among infertile men without clinical symptoms in our population. Here we estimate genital infection prevalence to other microorganisms and evaluate association between microbiological tests and seminal parameters alteration.

Methods: Forty patients attending to an infertility clinic, with ages between 27 and 51 (median 36 years) were studied. Semen analysis was conducted according to WHO indications and sperm morphology evaluated by Kruger’s strict criteria. Aliquots of semen were seeded in culture media for bacteria, fungi and yeasts. The statistical analysis was carried out using the Fisher’s exact test.

Results: Thirty-six patients (90%) showed alterations in their seminogram in one or more sperm parameters and 25 patients (63%) showed positive cultures. Fifty-six percent of cases were positive for Staphylococcus (S. aureus, S. epidermidis, S. cohnii, S. hominis, S. warneri and S. haemolyticus); 28% for Mycoplasma (M. hominis and M. fermentans), 8% for Streptococcus (S. pneumoniae and S. agalactiae), 4% for Candida albicans and in one patient (4%) Arcanobacterium haemolyticum was isolated. Association between positive microbiological tests and sperm parameters alteration or leukospermia was not observed. After receiving antibiotics therapy for those pathogenic microorganisms, only one patient (4%) infected with M. hominis return to fertility status.

Conclusion: These preliminary results suggest that in infertile men without symptoms of genital infection it is frequent to find pathogenic microorganisms that are not necessarily associated to seminal alterations, but could indicate a risk to cause female infertility to their partners.

P5/6 – 151

THE PREVALENCE OF BACTERIAL INFECTION IN THE SEMEN OF INFERTILE MEN.
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INTRODUCTION AND OBJECTIVES: To examine the prevalence of asymptomatic bacterial infection (asymptomatic, category 4 prostatitis) in the semen of fertile and infertile men. METHODS: Semen and urine (VB1) (VB2) cultures (aerobic, Chlamydiales) were performed on samples from consecutive non-azooospermic men presenting for infertility evaluation (n=209), fertile men presenting for vasectomy (n=60) and men submitting post-vasectomy samples (n=36). Men with positive cultures were treated with appropriate antibiotics and submitted a post-treatment semen sample. Standard semen parameters were recorded. RESULTS: 22% (46/209) of the infertile men had a positive semen culture compared to 7% (4/60) of the men presenting for vasectomy and 11% (4/36) of the men post-vasectomy (p<0.01). Only 4 men had urinary infections. The most common organisms were Enterococcus 38/46 (83%) and E. Coli 20/46 (43%). 3 of the infertile men had Chlamydia infection. Infertile men with a positive infection had significantly lower sperm concentration than those men with no infection (12.5 ± 1.4 vs. 19.2 ± 2.5, p<0.01). Appropriate antibiotic therapy resulted in eradication of the bacteria in only 87% (40/46) of cases although antibiotic therapy in these men did not improve semen parameters. CONCLUSIONS: Our data suggest that a significant percentage of men presenting for infertility evaluation have an asymptomatic genitourinary tract infection with what would normally be considered to be pathogenic bacteria. Given that the bacteria are often not found in the urine, in most cases, but found in the semen and in men post vasectomy, this suggests that the infection is likely in the prostate/seminal vesicle region. Antibiotic therapy can effectively eradicate the infection but has little effect on semen quality in the short term.
P5/6 – 152

GENITAL MYCOPLASMAS AND ITS IMPACT ON SPERM SAMPLES IN INFERTILE MEN.
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INTRODUCTION: It has been suggested the genital tract infections could be the cause or the agent of the male infertility. Microorganisms such as Ureaplasma Urealyticum and Micoplasma hominis generate subclinical genital infections or non gonococcal Urethritis in 25% of the infertile men. OBJECTIVE: To analyse the frequency of the seminal parameters alteration in infertile men with M. hominis and U. urealyticum infection. MATERIAL AND METHODS: Ninety nine urethral and seminal samples of the infertile men were studied by Semen analysis following the guidelines of WHO1999, to exception of morphology by strict criterios of Kruger. The mycoplasmas by special cultures. Statistical Analysis by Student ‘t’ test

RESULTS: Twenty four urethral and seminal samples presented genital mycoplasma infection: U.urealyticum was found in 15 and M. hominis was found in 9 cultures. Alteration of the seminal parameters in samples of infertile men with U.urealyticum and M. hominis infection PARAMETERS U. urealyticum n=15 M. hominis n=9 p < 0.05 VOLUMEN/ mL 3.35 ± 2.23 1.94 ± 1.60 0.04 SPERM/ 106 62.26 ± 64.29 29.55 ± 49.52 NS MOTILITY % (a + b) 30.8 ± 23.46 17 ± 17.61 0.05 VITALITY % 65.8 ± 20.19 46.44 ± 31.80 NS MORPHOLOGY % 5.93 ± 6.78 2.22 ± 2.90 0.03 The Mycoplasmas caused a decreasing quality of the sperm. The debris were present in all the samples. Those samples presenting U.urealyticum infection was found spermatozoas with a thin or very short flagellum in 20%. CONCLUSIONS: There is a major prevalence to the male infection of the genital tract by U.urealyticum than M. hominis. However, both microorganisms cause a decreasing quality of the sperm.

P5/6 – 153

GENOMIC ORGANIZATION OF THE SP22 GENE AND A UNIQUE PATTERN OF EXPRESSION IN SPERMATOGENIC CELLS. JE Welch*, RR Barbee*, JD Suarez*, NL Roberts*, and GR Klinefelter. Reproductive Toxicology Division, NHEERL, U.S. EPA, Research Triangle Park, NC, USA.

Our laboratory has reported that the amount of SP22 protein present on spermatozoa closely parallels the fertilizing ability of these cells. We have previously identified and sequenced two SP22 transcripts and demonstrated that expression of the rat SP22 gene produces a single 1.0 kb mRNA in somatic cells while both a 1.5 kb (SP22A) and a 1.0 kb (SP22B) transcript are present in the rat testis. Northern blotting of total RNA from the prepuberal rat testis indicated that while the 1.0 kb SP22B mRNA was expressed throughout development, the 1.5 kb SP22A transcript appeared coincident with spermatoocyte differentiation. Analysis of RNA from isolated spermatogenic cells confirmed that SP22A is expressed in pachytene spermatocytes and round spermatids. No SP22A transcript was detected in RNA samples from Leydig cells. SP22 has also been described as a contraceptive associated protein (CAP 1) encoded by a single transcript of 1.6 kb. A close examination of the CAP1 sequence (Genbank #AJ007221) suggests that this discrepancy is due to a fusion of SP22 and hepatoma derived growth factor transcripts within the CAP1 sequence. The SP22 segment of the CAP1 CDNA also contains a deletion of 51 bp in the 5’ untranslated region not found in the five SP22 clones sequenced. Products of the SP22 gene have been described in mammary as a putative oncogene (DJ-1) and as a RNA-binding complex (RS). Initial characterization of the human SP22 gene has identified 7 exons in 23,594 bp on chromosome 1. This abstract does not necessarily represent U.S.EPA policy.

PP – 001

ANDROGEN SELECTIVITY OF NATURAL DIRECT REPEAT RESPONSE ELEMENTS.
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Pem is a homeobox-type transcription factor mainly expressed in androgen-dependent tissues. Following chemical castration of mice, a dramatic down-regulation of Pem transcripts was observed in the epididymis. Using cell-based transactivation assays, two functional androgen response elements were found in the Pem promoter. Both elements differed from the classical palindromic motifs and exhibited features of direct repeats. Four copies were placed upstream of the TK minimal promoter and of the luciferase reporter gene for transfection studies. The proximal ARE-1 element displayed a high selectivity for androgen, as compared to glucocorticoid or progesterone stimulation. The distal ARE-2 was activated to a similar extent by androgen and glucocorticoid. In comparison, a consensus ARE was more responsive to glucocorticoid than to androgen treatment. Electroreptory mobility shift assays using in vitro translated androgen receptor showed ARE-1 and ARE-2 to form specific complexes. A supershift was observed in the presence of an anti-androgen receptor antibody. These results demonstrate that two ARES with direct repeat features participate in the androgen response of the Pem gene. Both display novel steroid selectivity profiles due to unique variations in their sequence. This selectivity might play an important role in vivo, especially with respect to differential gene regulation in normal tissues and in proliferative, androgen-dependent tumors.

PP – 002

ENDOTHELIAL CELL PROLIFERATION AND EXTRACELLULAR MATRIX PRODUCTION ARE INCREASED BY HYPERPLASTIC HUMAN LEYDIG CELLS. JI Regadera*, EF Alvarez-Ferreira, MP de Miguel, A Serrano*, P Gonzalez-Pereirato, M Nistal, and CA Suarez-Quian*, Dept Morphol & Pathol, Autonoma Uni Madrid, Spain; Kimmel Cancer Center. Thomas Jefferson Univ, Philadelphia; Dept Pathol & Urol, Guadalajara Univ Hosp; Dept Cell Bio, Georgetown Univ Med Cent, Washington, DC

Microvascular changes associated with seminiferous tubule (ST) and interstitial cell atrophy in cryptorchid testes (CT) are poorly characterized. Using immunohistochemistry and morphometry, we correlated microvessel involution and angiogenesis with nodular Leydig cell hyperplasia as a function of testosterone (T) immunostaining in 47 adult CT. Ten normal testes served as controls. Arterioles, capillaries and venules were quantified using endothelial CD34. Inginal CT were atrophic, exhibiting decreases in ST diameter (containing only Sertoli cells) but increases of the interstitium. A decrease of the peribular and interstitial capillaries (p < 0.01) and the arteriole-venules (p < 0.05) were found associated with an increase in both vacuolated (40%) and disgenetic (35%) Leydig cells. Only 5% of Leydig cells expressed normal T levels, while 75% showed a decrease and 20% were T negative. The abdominal CT exhibited complete tubular sclerosis and marked evidence of Leydig cell adenomatous hyperplasia, exhibiting strongly T-positive to T-negative staining. In both types of Leydig cell nodules, microvessel increase was observed and in some cases a pseudotumoral angiomatous transformation was found. The PCNA-labeling index was increased in both Leydig and endothelial cells. The hyperplastic Leydig cells presented an increased in the production of vimentin, actin, collagen IV, laminn, E-cadherin and β-catenin. One interpretation of these results is that there is a positive correlation between lack of Leydig cell activity and microvessel structural damage in the CT.
INJECTABLE TESTOSTERONE UNDECANOATE (TU) WITH ORAL OR INJECTABLE NORETHISTERONE (NET) PREPARATIONS FOR MALE CONTRACEPTION.


Introduction: Combined injections of 1000 mg TU and 200 mg NET enanthate (NETE) in 6-week intervals resulted in azospermia in most of the volunteers tested (1). This study was designed to test the efficacy of TU in combination with NETE or oral NET acetate (NETA) in a further phase II clinical trial. Subjects and Methods: 42 healthy men were randomized to receive: Group I) 200 mg NETE at study weeks 0, 6, 12 and 18 together with 1000 mg TU at study weeks 2, 6, 12 and 18; Group II) 400 mg NETE and 1000 mg TU at study weeks 0, 6, 12 and 18; Group III) 10 mg NETA daily and 1000 mg TU at study weeks 0, 6, 12 and 18. The treatment period (24 weeks) was followed by a control period of 28 weeks. During the study semen parameters, reproductive hormones, clinical chemistry, lipid parameters, well-being, sexual function as well as tests and prostate were monitored. Results: Marked suppression of gonadotropins in all groups resulted in azospermia in 13/14, 13/14 and 12/14 volunteers and severe oligospermia (< 1 million/ml) in the remaining volunteers in treatment groups I, II and III, respectively. With the exception of elevated T serum levels 2-4 weeks after injection, T concentrations during the whole treatment period remained within the normal limits. Conclusions: The combination of TU with NETE or NETA can be considered a first choice for further studies of male contraception. However, the higher NETE dose as well as the T-free interval offers no advantage over our previous study (1). 1) Kamischke A. et al., Intramuscular testosterone undecanoate and norethisterone enanthate in a controlled clinical trial for male contraception. J.Clin.Endocrinol.M

DELIVERY OF ANTIBODIES FOR MALE IMMUNOCONTRACEPTION.

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Although there are claims of immun contraception in males against reproductive antigens the claims are not supported by evidence that sufficient antibody can be delivered to the reproductive ducts to be effective. Consequently, we have assessed antibody delivery using microsurgical methods to collect luminal fluids from animals immunized with testis toxoid. Determinations of IgG in rats immunized subcutaneously showed that it is delivered in the secretions of the seminal vesicles and prostate gland respectively at 0.2% and 0.3% of the concentration in blood. It enters the lumen of the rete testis to achieve 0.7% the concentration in blood and is concentrated (due to fluid reabsorption 1:7-fold in the efferent ducts and a further 1:3-fold in the ductus epididymis). Reabsorption of serum IgG by the efferent ducts (by estradiol administration) did not significantly reduce reabsorption of IgG, and the ductus epididymis compensated for the reduced fluid reabsorption by the efferent ducts. Immunization protocols that raise mucosal and systemic responses were compared in the mouse. Although intranasal immunization achieved the highest titer of IgG in serum, subcutaneous immunization achieved the highest titer in the sperm ducts. Rectal immunization achieved the highest titer in prostatic fluid (50% of serum), and is interpreted as a local production. Nasal immunization produced the highest titer of IgA in the rete testis and prostate, the titers in the prostate being 3.4-times the titer in blood. Strong PCR product for phg primers were demonstrated in the prostate indicating the presence of secretory component. It is concluded that a better understanding of mechanisms may lead to immuno contraception against antigens in the sperm ducts.

TESTOSTERONE UNDECANOATE (TU) MAINTAINS SPERM SUPPRESSION INDUCED BY CYPROMERONE ACETATE (CPR) PLUS TU.

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Preliminary studies showed that lower hormonal load is needed to maintain than to induce sperm suppression. In this study we evaluate whether TU alone or combined with low dose CPR can maintain sperm suppression induced by higher doses of CPR plus TU. 24 subjects were treated for 12 weeks with CPR 20 mg/day + TU 1000 mg/6 weeks (suppression phase: S) and then randomly divided to receive TU 1000 mg/8 weeks plus CPR 20 mg/day (n=8 CPR-20) or plus CPR 2 mg/day (n=8 CPR-2) or plus PLACEBO (CPR-0) for 32 weeks (maintenance phase M-P). RESULTS: 24 subjects completed the S and 22 the M-P. Spermatogenesis was suppressed below 1 M/ml in all subjects by week 12 and remained suppressed until week 44 in all subjects, with no differences among the groups. Biochemical parameters did not significantly change at any time in any groups. CONCLUSIONS: 1. TU alone or combined with lower doses CPR maintain sperm suppression induced by higher dose CPR + TU 2. This regimen does not induce significant toxicological effects in any subjects over 44 weeks of administration 3. this hormonal combination represents a promising prototype regimen for male contraception.

CONGENITAL BILATERAL ABSENCE OF THE VAS DEFERENS (CBAVD): CLINICAL AND SEMINAL PARAMETERS, AND CFTR GENE MUTATIONS.

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Introduction: Since 1990, CBAVD was proposed to be a primary form of cystic fibrosis because of a high incidence of CFTR gene mutations in CBAVD patients. Methods: In 41 azoospermic men, diagnosis of CBAVD was supported by either unilateral (n=4) or bilateral (n=25) surgical exploration, TRUS evaluation (n=10) or impalpable vasa deferentia (VD) on physical examination (n=2). Every patient was submitted to a physical and sertic evaluation, and to CFTR mutations screening of 508, G542X, N1303K, 1717-1 (G to A), W1128X, G551D, R553X, E507, 621 + 1 (G to T), R1162X and R117H) and to polymorphism analysis of the CFTR intron 8 polyymidine tract (5T allele). 14 patients had a renal echography. Results: In 7 cases out of the 12 clinically present VD, a vas deferens was surgically present as a fibrous cord or a non permeable duct (7/54 = 13.5%). In the remaining cases, no vas deferens was surgically observed (5/47 = 10.6%). In 24.4% of the patients (10/41), neither CFTR mutation nor 5T allele were detected. Two of the 4 patients with unilateral renal absence had a CFTR mutation. The association of a semen volume <= 1.0 ml and a pH<7.0, observed in 6/4% (25/41) of the patients, was significantly correlated to the presence of at least one CFTR mutation OR = 5.3 (95% CI = 1.12-26.8). Conclusions: A present scrotal vas deferens does not exclude a CBAVD. CBAVD men with both sperm volume <=0.1 ml and pH<7.0 are at higher risk of CFTR mutation. CBAVD men with unilateral absent kidney must be screened for CFTR mutations before inclusion in Assisted Reproductive Technologies.

Abstrats – President’s Posters
Abstracts – President’s Posters

PP – 007

DAX-1 REPORTER TRANSGENE EXPRESSION IN MOUSE TESTES.

The DAX-1 gene encodes an unusual member of the nuclear hormone receptor superfamily which acts as a transcriptional repressor. DAX-1 plays a critical role during gonadal and adrenal differentiation since mutations of the human DAX-1 gene cause X-linked adrenal hypoplasia congenita associated with hypogonadotropic hypogonadism. To study regulation of DAX-1 gene expression, a transgenic mouse model was generated using a reporter vector consisting of a 4 kb fragment of porcine DAX-1 5' flanking DNA in front of a yellow fluorescent protein reporter gene sequence (pDAX.EYFP). This construction was used to generate 7 transgenic lines of mice via conventional pronuclear microinjection. Transgenic embryos were recovered and genital ridges dissected for expression studies of the transgene. Expression of fluorescence was detected in testis between 11.5-d.p.c to 19.5-d.p.c. and was associated with testicular cords. Testes of mice transgenic for pDAX.EYFP were recovered at different times postnatally. Yellow fluorescent protein expression was detected in a sub-population of seminiferous tubules by 3 weeks of the age, and in virtually all tubules in the adult. Immunohistochemical analysis using a commercial antibody generated against the GFP family of proteins showed that transgene expression was restricted to the meiotic stages of germ cells. Presence of the reporter protein in all stages of embryonic testicular development and the meiotic stages of germ cells in seminiferous tubules suggests an important role of the DAX-1 gene in testis development and spermatogenesis.

PP – 008

THE NATRIURETIC PEPTIDES STIMULATE STEROIDOGENESIS IN THE FETAL RAT TESTIS.
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To study the regulation of fetal testicular steroidogenesis in the rat, we examined effects of members of the natriuretic peptide (NP) family, i.e. atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) on testosterone production by dispersed Leydig cells of rat fetuses of embryonic day (E) 18.5. All three natriuretic peptides stimulated testosterone production, with significant effect at concentration of *1 x 10-8 mol/L of ANP, *1 x 10-9 mol/L of BNP and *1 x 10-6 mol/L of CNP. RT-PCR analysis could not reveal ANP mRNA in the fetal or neonatal testes, while BNP and CNP mRNAs were detected in fetal testes as early as E15.5. Likewise, receptors for all three natriuretic peptides, i.e. NPR-A, NPR-B and C-receptors, were expressed in the fetal testes as early as E15.5. ANP was detectable in circulation and testis tissue by RIA on E18.5 and day 1 postpartum. The natriuretic peptides had no effect on cAMP production by fetal Leydig cells. When tested in combination with two other peptides shown before to stimulate fetal testicular steroidogenesis, adenylyl cyclase-activating polypeptide (PACAP-27) and vasoactive intestinal peptide (VIP), the combined effects did not differ significantly from the maximum effect of any one of the peptides alone. In conclusion, our present findings provide both functional and molecular evidences for the presence of NPR-A, NPR-B and NPR-C in the fetal testis. Taking together with the presence of ANP in the fetalplasma and the expression of BNP and CNP in the fetatlesis support the physiological significance of these findings and indicate the involvement of the natriuretic peptides in the endocrine and paracrine regulation of the early phase of fetal testicular steroidogenesis at the age of E15.5-E19.5, i.e. before the onset of pituitary LH secretion.

PP – 009

A NOVEL MUTATION IN THE D-BOX OF THE ANDROGEN RECEPTOR GENE (S597R) IS ASSOCIATED WITH BOTH PAIS AND NORMAL PHENOTYPE.
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Testosterone and 5α-dihydrotestosterone (DHT) both act through the androgen receptor (AR), which is encoded by a single copy gene located at chromosome X. Deficient function of the AR causes androgen insensitivity syndrome (AIM), characterized by varying degrees of undermasculinization in 46,XY individuals. The AR is divided into three main functional domains: an N-terminal domain involved in transcriptional activation of androgen responsive genes, a DNA-binding domain (DBD) and a steroid-binding domain. The DBD is commonly referred to as zinc fingers. The so-called proximal box (P-box) of the first zinc finger seems to give rise to complete AIS, whereas the distal box (D-box) in the second zinc finger has been associated with milder forms. We have identified a variant (S597R) in the D-box of the AR gene in two unrelated males. The first subject was a newborn 46,XY male diagnosed with partial AIS. Ile had one palpable testes, a small vaginal cavit, and perineocrotal hypospadias. An hCG test resulted in a rise in serum testosterone (T) from 2.2 to 8.8 nM and a swelling of the genitals was noted. Treatment with 1% DHT ointment was initiated. The other subject was a phenotypically normal man with normal sperm output (208 mill/ml) and hormone values within normal range: T 18.7 nM, FSH 1.8 nM, LH 2.3 IE/L, SHBG 27 nM, and Estradiol 115 PM. In childhood he had bilateral cryptorchidism, but both testes descended spontaneously. Thus, the same mutation at this codon can cause different phenotypes as shown by the variation in masculinization of these individuals, with one severely affected child and one normally developed man.

PP – 010

OXYTOCIN INCREASES ACTIVITY OF BOTH ISOFORMS OF 5ALPHA-REDUCTASE IN THE MOUSE EPIDIDYMIS.
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Dihydrotestosterone (DHT) is the major biologically active androgen in the epididymis. Recent work has shown that activity of the enzyme 5alpha-reductase, which converts testosterone to DHT, can be increased by the peptide oxytocin. Oxytocin is produced within the epididymis and oxytocin receptors are present on the epididymal epithelial cells. We have used 2 colonies of transgenic mice to investigate the effects of oxytocin on the 2 isoforms (types I and II) of 5alpha-reductase. One colony (3.5) over-expresses the bovine oxytocin gene in the hypothalamus resulting in increased circulating levels of oxytocin, whilst 4.2 transgenic mice only over-express the oxytocin gene in the male reproductive tract resulting in higher local concentrations of the peptide. The activity of both isoforms of 5alpha-reductase in adult mice epididymides was determined by measuring the conversion of 3H testosterone to 5alpha-reduced steroids at pH 7 (type I) and pH 5 (type II). Activity for both isoforms of the enzyme was detected in the epididymides of wild type mice with type I activity being almost twice that of type II. Both type I and type II activity was increased in the transgenic mice. Activity for both isoforms was nearly doubled in the 3.5 transgenic mice. In the 4.2 mice type 1 activity was increased by a factor of 7 whilst type II was only increased by a factor of 2.4. These data suggest that oxytocin may have a dose-related effect on 5alpha-reductase activity and that increased local concentrations of the peptide may preferentially increase activity of the type I isoform of the enzyme.
Abstracts – President’s Posters

PP – 012
IMMORTALIZED EPIDIDYMAL CELL LINES FROM TRANSGENIC MICE HARBOURING TEMPERATURE-SENSITIVE SV 40 LARGE T ANTIGEN GENE.

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Epididymal epithelium is well known as a productive site of various factors present in epididymal luminal fluid. Although there have been many reports of primary cultures of epididymal epithelial cells, their growth is limited in time. Therefore, at present, no cell line is available for the study of the regulatory mechanisms of epididymal function. We have established epididymal epithelial cell lines from temperature-sensitive SV 40 large T-antigen gene transgenic mice. These immortalized cell lines [1] from initial segment; DC1, DC2 and DC3 from distal caput] show temperature-dependent growth. Expression of cytokearin, a marker of epithelial cells, and phosphorylated ethanolamine binding protein, a marker of murine epididymal epithelium. A regulated epididymal protein (murine epididymal retinoic acid binding protein: mE-RABP) is also expressed in DC1 cells but the level of expression is low. The androgen-regulated 5kb mE-RABP promoter DNA fragment ligated to neomycin resistant gene was used for stable transfection of DC1 cells. Since the mE-RABP gene is specifically expressed in the distal caput, neomycin-selection provides a pure population of epithelial cells from that segment. This neomycin-resistant immortalized cell line from the distal caput has been cultured for more than 6 months. Such neomycin-resistant cell lines should be valuable tools to study the regulation of tissue specific gene expression, and may be used to identify epididymal specific transcription factor(s) involved in the expression of epididymal specific proteins. (Supported by the Rockefeller/Emst Schering Foundations and NIH:HD36900)

PP – 013
EPIDIDYMIS-SPECIFIC SPERM BINDING PROTEINS.

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To increase our understanding of the molecular basis of sperm maturation, we prepared a Macaca mulatta epididymis cDNA library subtracted against testis. The library contains 31 novel cDNAs. Epididymis-specific expression was verified by Northern hybridization to RNAs from 24 organs. The 31 include several predicted protein modifying enzymes and inhibitors that could activate or protect sperm surface proteins. SCA2 contains a trefoil motif and was selected for further study based on abundance. The mRNA for SCA2 is androgen-regulated as determined by Northern hybridization to monkey epididymis RNA from normal androgen withdrawn and testosterone replaced animals. SCA2 is located on the surface of human ejaculated sperm where a function in promoting fertilizing ability is possible. Support for this project [CIG96-06-A] was provided by the CICCR Program of the Contraceptive Research and Development Program, Eastern Virginia Medical School, the National Institutes of Health grant HD04466 (FSF). The Andrew W. Mellon Foundation and by NICHD/NIH through cooperative agreement U54 HD35041 as part of the Specialized Cooperative Centers Program in Reproduction Research.

PP – 014
HOX TRANSCRIPTION FACTORS AND SEGMENTAL FUNCTION OF THE ADULT EPIDIDYMIS.

Daniela Bomgardner, Barry T. Hinton, Terry T. Turner. Depts of Urology and Cell Biology, Univ. of Virginia, Charlottesville, VA. Previous studies show that the adult mammalian epididymis is highly segmented in structure and function. Developmentally, the formation of segmented structures is regulated by hox genes. Certain hox genes have been detected in developing epididymides. We hypothesize that hox genes are present and are involved in segmental function of the adult epididymis. The epididymes of adult C57BL/6 mice were divided into initial segment, caput, corpus, cauda and vas deferens. RT-PCR revealed the expression of hoxa-9, -10, -11, and hoxd-9 and -10. Hoxa-11 appeared in a regionalized expression pattern, confirmed by Northern blot analysis. Highest expression was observed in the distal region compared to the proximal epididymis. Western blot analysis showed that both hoxa-10 and a-11 are probably translated as well. These proteins are known to be transcription factors that form DNA-binding complexes with specific cofactors, such as Meis 1. RT-PCR detected both transcripts of Meis 1, 1a and 1b, in the adult mouse epididymis. Potential downstream targets of hox genes are cell adhesion molecules (CAMs), which are pivotal for the maintenance of three-dimensional structure of organs. RT-PCR was used to examine the expression of NCAM and L1-CAM mRNAs in the epididymis. Both genes were found to be expressed, with L1-CAM showing a higher expression in the distal region compared to the proximal epididymis. These results indicate that hox transcription factors might be important for adult epididymal structure and function. Supported by T32HD07382 and DK14579
Abstracts – President’s Posters

PP - 015

DIFFERENTIAL REGULATION OF GENE EXPRESSION ALONG THE EPIDIDYMIS AFTER ORCHIDECTOMY.
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The epididymis is the site where spermatozoa acquire motility and fertilization potential. Within 12 hours after orchidectomy, serum androgens decline by more than 95%, whereas clearance of luminal fluid from the epididymis does not occur over a 7 days period. We hypothesize that gene expression is differentially affected in the caput and cauda epididymides at 2-days post-orchidectomy.

Methods: RNA from the caput and cauda epididymides of control or 2-day orchidectomized (ORC) Brown Norway rats was used to probe Clontech Rat Stress Toxicology II cDNA arrays (n=5/group) and data were analyzed with GeneSpring™ software. Changes in gene expression of at least 2-fold are discussed.

Results: In the caput epididymidis, there was an increase in the number of detected genes from 203 in control to 251 genes at 2-days post-ORC. Of the 86 genes that changed in this segment at 2 days post-ORC, 80 increased; many were apoptosis-associated genes or members of the heat shock family. In the cauda epididymidis, there was a decrease in the number of detected genes from 233 in control to 212 genes at 2-days post-ORC. Of the 99 genes that changed in this segment 2-days post-ORC, 91 decreased, including metabolism and bcl-family genes.

Discussion: Overall up-regulation of several genes in the caput epididymidis supports an active tissue response at 2 days post-orchidectomy; in the cauda epididymidis, not yet of luminal fluid at 2 days post-ORC, down-regulation of several genes is likely to be the result of androgen-withdrawal alone. We suggest that the rate of clearance of luminal fluid is responsible for differential gene expression changes observed in the caput and cauda epididymides at 2-days post-ORC. Supported by NIH AG08321 and CHIR.

PP - 016

EVENING MELATONIN FURTHER INCREASES ADULT TESTES SIZE AND GERM CELL NUMBER IN NEONATAL HYPOTHYROID RATS.
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Hypothyroidism induced in neonatal rat pups by 6-Propyl-2-thiouracil (PTU) feeding of mother through drinking water from day 1 to day 21 of postnatal life (preweaning period) increased adult testes size at 90 days by 47 % irrespective of the maintenance photoperiod (LD 8 : 16 in the current study). This is accredited to prolonged Sertoli cell proliferation during the neonatal period as thyroid hormone is credited with the role of inducing Sertoli cell differentiation. In another experiment neonatal rat pups were administered melatonin (40 mg/animal) from day 1 to day 21 in the evening at 16.00 hrs and exposed to lights off. These animals showed puberty enhancement as marked by hastened spermatogenesis with appearance of sperms for 45 days as against 60 days in controls. In the adult stage, the germ cell population in the tubules was significantly high and more compactly packed as seen in histological sections. The testes weight was also 12 % heavier than in the control. The serum hormone profile of melatonin treated rats showed higher Concentrations and T3 levels till 35 days. Though the T3 level remained highest throughout, Concentrations level became subnormal in the adult condition. As per our previous inference, melatonin induced increase in germ cell number is correlated with an indirect action mediated by Corticosterone in decreasing germ cell apoptosis relative to the control. This action of Corticosterone is purported to be through its action on Sertoli cell in modulating secretion of appropriate paracrine factors by way of altered gene expression. A third experiment was set up to test the effect of a combination of hypothyroidism and melatonin.

PP - 017

HIGH INCIDENCE OF SINGLE NUCLEOTIDE SUBSTITUTIONS IN THE MITOCHONDRIAL GENOME IS ASSOCIATED WITH POOR SEMEN PARAMETERS IN MAN.
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Single nucleotide polymorphisms (SNPs) in 7000 bp of the mitochondrial genome, encompassing 15 coding regions from COI to ND5, were characterised by single strand polymorphism analysis and confirmed by DNA sequencing. About 4% of normozoospermic men and 8.4% of the men with poor semen parameters had at least one nucleotide substitution. Most of the substitutions occurred in the third codon and did not change the amino acid. Hydrophobicity plots of the mutations with changes in an amino acid as a result of a nucleotide substitution suggested that they did not affect the function of the protein. The two most common substitutions at nt 9055 and 11719 had significantly higher frequencies in men with reduced sperm motility. Eleven percent of the men with poor semen parameter and 1.3% of normozoospermic men had a 9055 substitution, 12% of the men with poor semen parameters had a substitution at nt 11719, but none of the fertile men had this substitution. All the patients with these substitutions had reduced sperm motility and/or low sperm count. These SNPs in the mitochondrial genome were in a homoplasmic state, and, thus, were likely inherited from the mothers of these men. We propose that the semen quality of these men may be comprised through the inheritance of mutated mitochondrial DNA from their mother.

PP - 018

PREDICTING PREGNANCY AND SPERMATOGENESIS BY SURVIVAL ANALYSIS DURING GONADOTROPIN TREATMENT OF GONADOTROPIN DEFICIENT INFERTILE MEN.
PV Liu, L Turner*, A Comway*, S Wishart* and DJ Handelsman, ANZAC Research Institute and Department of Andrology, Concord Hospital, University of Sydney, Sydney NSW 2139, Australia.

Predictors of fertility or spermatogenesis during gonadotropin therapy of gonadotropin deficient infertile men are poorly defined. To evaluate potential predictors and compare recombinant with urinary FSH, we analyzed 43 consecutive courses of therapy given to 29 gonadotropin-deficient men (20 with hypothalamic disease) desiring paternity in a single centre. The Kaplan-Meier estimate of median time to a sperm concentration of >0, >5 and >20 M/mL were 5.5 (SE 1.1), 12.4 (SE 2.3) and 29.1 (SE 1.9) months respectively. Conception occurred in 22/43 cycles (8 men producing two pregnancies) with a Kaplan-Meier estimate of 20.5 (SE 4.7) months. The median sperm density at conception was 5.0 (SE 2.0, range 0.0-59.5) M/mL. Multivariate Cox proportional hazards models predicting these sperm thresholds and conception were developed by stepwise regression variable selection. Larger tesis volume, prior gonadotropin therapy, completion of puberty, the absence of adverse fertility factors or multiple pituitary hormone deficiency, but not age nor prior androgen therapy, predicted favorable response. Multivariate modeling identified the two most important predictors of sperm output as testicular volume (P<0.005) and completion of natural puberty. The efficacy of recombinant and urinary FSH were similar. The importance of spontaneous puberty and prior gonadotropin therapy suggest possible advantages to inducing puberty with gonadotropin rather than androgen therapy in gonadotropin deficient adolescents. The prolonged time required to induce spermatogenesis indicates the desirability of using these predictors to facilitate appropriate early use of reproductive technologies.

We previously found that levels of SP22 are highly correlated with fertility. Herein, we sought to: 1) describe localization of SP22 in rat testes, epididymis, and sperm; 2) demonstrate inhibition of fertility of rat sperm in vivo and in vitro; and 3) identify linear epitopes. Polyclonal antisera was raised in sheep against full-length recombinant SP22 (rSP22). Hybridoma clones were generated from mice immunized with rSP22 and boosted with native SP22. Sperm clones were used for ascites production. Immunoblots indicated that rSP22 Ig and ascites recognized denatured and native SP22, respectively. Cytoplasm of pachytene spermatocytes and round spermatids were immunostained with both rSP22 Ig and ascites. In stage VIII, staining was concentrated along the spermatid head. Isolated retic testis sperm revealed discrete staining at the base of the head, staining migrated to the equatorial segment of the head as sperm moved to the cauda epididymis. Interestingly, clear cells were immunostained along the length of the epididymis. Ascites, rSP22 Ig, and rSP22 Fab, each exhibited fertilization in vivo and in vitro. For example, rSP22 Ig (1:50) added to cauda epididymal sperm 5 minutes prior to in utero insemination decreased fertility from 74% to 34%, and SP22 Ig (1:10) added to sperm incubated overnight with eggs decreased fertilization from 81% to 39%. Linear epitope mapping of the 189 amino acid SP22 sequence revealed three distinct sequences recognized by rSP22 Ig and one sequence recognized by ascites. Current work is focused on the potential role of these sequences in sperm-egg interaction.


Introduction: Sildenafil represents a powerful therapy for male erectile dysfunction. Anecdotally, some young healthy males who wish to enhance their sexual performance are requesting or abusing sildenafil. In this study, we investigated sildenafil efficacy in young men without erectile dysfunction in a randomized double-blind, placebo controlled clinical study.

Material and Methods: 92 young healthy men, all volunteers, age 20-40 without erectile dysfunction evaluated with the IIEF questionnaire were enrolled. Subjects were not using any medication for the 6 month period prior to the study and had also been engaged in a 3 month stable relationship. 60 were enrolled for the study. They all signed a consensus agreement. They were divided following a randomized double-blind comparison in two groups of 30. Group I was prescribed one tablet of sildenafil of 25 mg and Group II a placebo tablet. All the subjects have filled a questionnaire.

Results: There were no differences between the two groups in the improvement of the erection parameters 1/30 sildenafil vs 1/10 placebo while side effects were more frequent in sildenafil group 5/30 vs 1/30. Interestingly, sildenafil administration led to a marked reduction of the post-ejaculatory refractory time in the presence of a continuous erotic stimulus 10/30 vs 4/30.

Conclusions: Sildenafil does not improve erections in young healthy men. Even in the lowest prescribed dose it has significant side effects in this group. Sildenafil should not be given to young healthy men to improve their erections. The reduction of the post-ejaculatory refractory time suggests that there may be a place for studying its use in some men with premature ejaculation.


In the last few decades, concern has been growing about the threat to male reproductive health from chemical and physical compounds released into the environment. However, less attention has been paid to their impact on male sexual function than to their effect on male fertility. We investigated the relationships between exposure to environmental agents and the ability to achieve and maintain an erection within 175 consecutively men consulting for organic erectile dysfunction (ED) between 1995 and 1998, in the Litoral Sur region of Argentina. Nocturnal penile rigidity and tumescence were simultaneously and continuously monitored during two consecutive nights, using a RigScan device. Men were classified as having incomplete ED or complete ED according to the classification patterns of Yasumoto et al. (Hinyokika Kyio, 42,285-8,1996). The association of environmental exposures with ED was estimated by logistic regression and adjusted for potential confounders. We observed a significantly higher risk of complete ED than for incomplete ED for those exposed to pesticides (OR: 3.4; 95%CI: 1.2-10.1) and solvents (5.4; 1.3-21.8). These factors remained statistically significant after adjustment for age, smoking and alcohol consumption. Our observations suggest that further prospective studies are required into the potential implication of chemical environmental factors in ED.
Abstracts – President’s Posters

PP – 023
EX VIVO EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RECEPTORS IN HUMAN PENILE CAVERNOSAL CELLS. Mahadevan Rajasekaran, Armen Kasyan*, Manoj Monga. UCSD Medical Center, San Diego, CA.
Vascularogenic erectile dysfunction is associated with impairment of vascular perfusion to the erectile components of the penis. Vascular endothelial growth factor (VEGF) has been identified as the angiogenic permeability-inducing factor. The role of this factor in penile tissue vascularity is not well understood. This study evaluates the ex vivo expression of VEGF and its receptors (flt-1, KDR) in human penile cavernosal smooth muscle cells (HSCMC) in order to explore the therapeutic strategies for improving penile tissue vascularity.
Primary culture was initiated with explants of human corpora cavernosa and early passage (3-5) cells were used for these evaluations. Identity of smooth muscle cells was established by immunohistochemical assays for smooth muscle specific α-actin (anti-α-SMA; DAKO Labs). Endothelial cell contamination was checked using a specific marker (von Willebrand factor) by avidin-biotin complex technique. To evaluate gene expression of VEGF, flt-1 and KDR, total RNA was extracted (TRIZOL reagent) from cavernosal cells and subjected to reverse transcription-polymerase chain reaction (RT-PCR) using custom synthesized (GIBCO-BRL) primers.
Immunohistochemical studies confirmed the presence of smooth muscle cells in these cultures that were devoid of endothelial cell contamination. The RT-PCR evaluation revealed the expression of four splice variants of VEGF mRNA (VEGF 120,144, 164, 188) and two of its receptors (flt-1, KDR) in HSCMC. VEGF 164 was the most abundant form of mRNA whereas flt-1 appeared to be the most prominent receptor type in these cells.
These findings suggest that human cavernosal smooth muscle cells express both VEGF and its receptors, which may be important in the control of vascularity in the penile architecture.

PP – 024
REGULATION OF RHO-KINASE MEDIATED VASOCONSTRICTION IN THE PENILE CIRCULATION. T Mills*, H Branan, K Chitaley, P Jackson, R Lewis, V Stopper, C Webb, C Wingard. Departments of Physiology and Surgery (Urology). Medical College of Georgia, Augusta, GA.
We recently reported (Nature Medicine 7:119, 2001) that inhibition of Rho-kinase increased the erectile response (intracavernosal pressure/mean arterial pressure, ICP/MAP) by a NO-cGMP independent pathway. The present study was undertaken to determine if the vasconstrictor agents which are active in the penis, act via the Rho-kinase pathway. Western analysis revealed considerable RhoA and Rho kinase protein in the penis. Inhibition of Rho-kinase with Y-27632 (specific Rho-kinase antagonist, Welfide Corporation, Osaka, Japan) caused a significant increase in the erectile response (Cont 0.06 ± .01; Y 0.80 ± 0.3). Intracavernous administration of endothelin-1 (ET-1, 50 pmol) or the α-adrenergic agonist, methoxamine (METH, 10 μg/kg) resulted in a marked reduction in electrically induced ICP/MAP (Cont 0.78 ± 0.5; ET-1 0.32 ± 0.1; METH - 0.23 ± .06). If, however, Y-27632 was given before ET-1 or METH, ICP/MAP remained elevated (Cont 0.86 ± 0.2; Y+ET-1 0.90 ± 0.3; Y+METH 0.83 ± 0.05). Contrariwise, when given after METH, Y-27632 had a reduced vasodilatory effect (Cont 0.23 ± .06; METH+Y 0.71 ± 0.08) or no vasodilatory effect when given after ET-1 (Cont 0.32 ± .01; ET-1+Y 0.31 ± .01). These findings suggest that ET-1 and METH increase the Rho-kinase activity in the cavernous circulation thus reducing the effectiveness of the Rho-kinase inhibitor. Our findings support the hypothesis that the vasocostriction which maintains the penis in the non-erect state is mediated, in part, by the Rho-kinase pathway.

PP – 025
EPIGENETIC CHANGES OF ANDROGEN RECEPTOR GENE IN HUMAN PROSTATE CANCER. H Suzuki, T Ichikawa, K Akahura, T Igarashi and H Ito, Department of Urology, Chiba University School of Medicine, Chiba, Japan; T Nakayama, M Watanabe and T Shiraiishi, Second Department of Pathology, Mie University School of Medicine, Mie, Japan.
It is generally accepted that epigenetic mechanisms including DNA methylation and histone acetylation/ deacetylation play important roles in gene transcriptional inactivation. Heterogenous expression of androgen receptor (AR), which appears to be related to variable responses to endocrine therapy in prostate cancer (PCa) may also be due by epigenetic factors. The methylation status of CpG islands of the AR gene promoter in 3 prostate cancer cell lines (DU145, TSU-P1 and LNCaP), 10 primary untreated PCa samples and 14 hormone-refractory PCa samples using the bisulfite-PCR methods. In DU145 cells, CpG rich regions of the AR were observed to be hypermethylated. By immunohistochemical study, only one PCa sample had no AR expression, the others being heterogenous. Bisulfite-direct sequencing and methylation-specific PCR analysis showed aberrant methylation of AR 5′ regulatory region in 2 of 10 (20%) primary and 4 of 14 (28%) hormone-refractory PCa samples. To clarify the influence of epigenetic regulation on AR gene expression, we treated 3 prostate cancer cell lines with a demethylating agent (5-aza-2′-deoxycytidine: Aza-C), and a histone deacetylase inhibitor (Trichostatine A: TSA). In DU145 cells, re-expression of AR mRNA could be detected after treatment with Aza-C and/or TSA. From these results, we concluded that epigenetic regulations including CpG methylation and histone acetylation may play important roles in the regulation of the AR.

PP – 026
SELECTIVE MOLECULAR ABLATION OF EPITHELIAL TISSUES IN CANINE PROSTATE: DEVELOPMENT OF PROSTATE-SPECIFIC ANTIGEN PROMOTER-BASED SUICIDE GENE THERAPY FOR BENIGN PROSTATIC HYPERPLASIA. J Cheon, HS Park, DG Moon, SK Koh, JJ Kim, Korea University College of medicine, Seoul, Korea
INTRODUCTION AND OBJECTIVES: To develop a novel gene therapeutic modality for the effective treatment of BPH, we investigated the properties of toxic gene therapy utilizing prostate-specific antigen (PSA) promoter driving herpes simplex virus thymidine kinase(HSV-TK) gene in canine prostate. METHODS: A replication-defective recombinant adenoval vector containing HSV-TK gene under transcriptional control of long PSA promoter (Ad/PSA/HSV-TK) was developed. The adenoviral vectors were delivered to the anesthetized adult beagle dogs by intraprostatic injection on day 1 and 7 with subsequent prodrug acyclovir (ACV) administration intravenously for 14 days. They were injected into only left half of the prostate and untreated right half was used as control. After 2 weeks, animals were sacrificed and prostates were harvested and sectioned. Therapeutic efficacy was determined by histological changes, apoptosis (TUNEL assay) and immunohistochemical staining for PSA. RESULTS: Injection of canine prostate with Ad/PSA/HSV-TK led to striking apoptosis of epithelial cells on TUNEL assay. On immunohistochemical studies there were markedly decreased number of PSA-secreting epithelial cells of Ad/PSA/HSV-TK treated left half of prostate compared to control. Histomorphological evaluation also demonstrated significant atrophy of prostate glands in treated side. No treatment related death of animals was observed during this study. CONCLUSIONS: The PSA promoter-based suicide gene therapy induced highly selective and definite ablation of epithelial tissues in canine prostate. Our novel approach could open opportunity of gene therapeutic modality for the treatment of clinical benign prostatic hyperplasia.
PP – 027
PROSTATE AND TESTIS ARE RICH SOURCES FOR GLYCOPROTEIN HORMONE SUBUNITS IN HUMAN SEMEN.
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Testicular function is predominately regulated via the hypothalamic-pituitary-testicular axis, whereby the glycoprotein hormones (GPH) as well as testosterone are major endocrine or local regulators. It has now been investigated whether GPH and the free α and β subunits thereof are produced in the target organs themselves and potentially act as auto/paracrine modulators of fertility. RT-PCR, Northern blotting, immunohistochemistry (IHC), Isoelectric focusing, Western blotting and IFMA - IHC, Western blotting and IFMAs based on our panel of highly selective monoclonal antibodies - have been utilized for the detection of human chorionic gonadotropin (hCG) and its free subunits in the human testis, prostate and seminal plasma. It appeared that both organs synthesize hCGα and hCGβ which are then found in high concentrations in seminal plasma of healthy prostates (n=17): hCGα 2.6±0.5 μg/ml (mean±SEM), hCGβ 2±0.28 ng/ml and hCG 190±39 pg/ml. All these parameters dramatically exceed physiological values, e.g. ten-thousand-fold in the case of hCGα, in serum of young men (n=20): hCGα 142±54 pg/ml (mean±SEM), hCGβ 50 pg/ml and hCG 4±3 pg/ml. Neither of these markers is correlated to sperm counts. Seminal plasma is the richest physiological source in both genders for genuine free i.e. non-dissociated hCGα. Its concentration is as high as in maternal serum (weeks 10-12 of gestation) and in extra-embryonic coelomic fluid. It is not derived from heterodimeric GPH3 pointing to hCG-independent functions of hCGα and hCGβ in male fertility. Austrian Science Funds (P13652-GEN).

PP – 028
LIGAND-INDEPENDENT ACTIVATION OF THE ANDORGEN RECEPTOR AND THE ROLE OF THE COACTIVATOR SRC-1a IN PROSTATE CANCER CELLS.
Introduction: Progression of prostate cancer to androgen independence may involve recruitment of non-androgenic pathways of activation of the androgen receptor (AR). The AR can be activated in the absence of androgens by interleukin-6 (IL-6) in LNCaP human prostate cancer cells. Recently, coactivators that physically interact with the AR have been identified. Here, we investigated whether steroid receptor coactivator-1a (SRC-1a) altered transactivation of the AR when the AR was activated in the absence of androgens in LNCaP cells exposed to IL-6. Methods: LNCaP cells were transiently cotransfected with AR-driven reporter plasmids and an expression vector for SRC-1a. Cells were exposed to the synthetic androgen R1881, IL-6, or the MEK inhibitor U0126. Transactivation assays were performed in cells cotransfected with expression vectors of AR1-558Glu4DBD, the Gal4-luciferase reporter, and SRC-1a. prior to treatment with IL-6 or vehicle. Co-immunoprecipitation studies were performed to evaluate interactions between the AR1-558 and SRC-1a in cells treated with IL-6. Results: Ectopic SRC-1a enhanced the activities of AR-driven reporters induced by IL-6. U0126 inhibited the activities of AR-driven reporters induced by R1881 and IL-6, both in the presence and absence of ectopic SRC-1a. Synergistic increases in Gal4-luciferase activities (measures activation of AR1-558) were dependent upon the concentration of SRC-1a in cells exposed to IL-6. Protein-protein interactions between SRC-1a and AR1-558 were detected in IL-6 treated cells. Conclusion: SRC-1a enhances transactivation of the unliganded AR in the presence of IL-6 which may involve protein-protein interactions between SRC-1a and amino acids 1-558 of the AR.

PP – 029
DOES CASODEX INDUCE HORMONE-DEPENDENT PROSTATE CANCER CELLS TO BECOME INVASIVE?
Marin Teniswood, Ping Zhan, Jennifer Walker, Chun Yu Lee, and Kathryn Packman*. University of Notre Dame, Notre Dame, Indiana, USA.
Antiandrogens have traditionally been utilized for treatment of prostate cancer in the case of disseminated disease or as an adjunct to surgery. During the last ten years, antiandrogens have been increasingly used in the clinic for the treatment of localized prostate cancer. In addition, antiandrogens are now being considered as a chemo-preventative therapy for patients at high risk for developing prostate cancer. We have shown that treatment of hormone-dependent, non-metastatic LNCaP cells with the antiandrogen casodex induces an invasive phenotype in a small but clinically significant proportion of cells that survive treatment. This has lead us to hypothesize that the invasive phenotype observed in a small sub-set of cells treated with anti-androgens might in fact be the result of abrogated cell death. If apoptosis, and cytoplasmic condensation is initiated, but DNA fragmentation is abrogated in a significant number hormone resistant cells, the surviving hormone resistant cells, which over-express extracellular matrix proteases may acquire a more aggressive phenotype, with increased metastatic potential. The invasive cells have been clonally expanded to generate a casodex-resistant sub-line which has altered morphology and growth characteristics compared to the parental non-invasive cells. The sub-line has retained invasive potential, suggesting that acquisition of the invasive phenotype is not epigenetic. Our results suggest that the short-term benefits of using anti-androgens may not out-way the negative longer-term effects, especially for patients with aggressive disease. This work was supported by the Coleman Foundation.

PP – 030
WITHDRAWN
Abstracts – President’s Posters

PP – 031

KINESIN LIGHT CHAIN EXPRESSION IN SPERMATIDS.
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We isolated a novel kinesin light chain, KLC3, from a rat testis cDNA library. The KLC3 gene is linked to ERCC2 on human chromosome 19q13.2-13.3. KLC3 contains the typical heptad repeat and tetratrico-peptide repeats identified in other KLC proteins. RT PCR analysis of the expression pattern of KLC3 in testis shows that whereas KLC1 (neuronal) and KLC2 (ubiquitous) are expressed in premeiotic cells, KLC3 is only expressed after meiosis in spermatids as a 59,000 protein in western assays. Immunocytochemical detection confirms the mRNA pattern: KLC3 expression is first detected in step 10 spermatids, peaks at step 15-16 and diminishes again at later stages of differentiation, in apparent association with the axoneme and outer dense fibers (ODF) in the sperm midpiece. Immunofluorescence detects the protein in the tail midpiece of elongating sperm. The sperm tail association is maintained shortly after fertilization of eggs. Using a yeast 2-hybrid system we show that KLC3 can interact with Odf1, but not with Odf2, and that the leucine zipper is involved. The ODF association was confirmed in a biochemical assay using purified ODF. In vitro, KLC3 can associate with microtubules (MT) isolated from brain and tests in agreement with its possible interaction with the axoneme; however, in vivo KLC3 does not associate with MT of the manchette. Our data suggest a distinct role for KLC3 in post-meiotic testicular cells.

PP – 032

GERM CELL TRANSPLANTATION IN HR6B KNOCKOUT MICE.
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Inactivation of the HR6B gene in mouse causes male infertility. In these knockout mice spermatogenesis appears to be initiated normally, but during post-meiotic development impairment of spermatogenesis becomes evident, resulting in the production of a low number of spermatozoa with a highly abnormal morphology. In some animals, a few normal spermatozoa can also be found. It has been hypothesized that this impairment of spermatogenesis is due to a defect in the ubiquitin system in the germ cells. An alternative explanation could be a deficient functioning of somatic testicular cells, in particular the Sertoli cells. To address this question, we have transplanted germ cells of mice transgenic for the lacZ gene (ROSA26) into the testis of HR6B knockout mice. At least one month before transplantation the knockout acceptor mice were treated with busulphan. The donor mice were made cryptorchid, to increase the proportion of spermatogonial stem cells in the testis. Three months after transplantation, tubuli of the testis of knockout mice were found to be colonized by b-galactosidase expressing germ cells. Histologically, some tubuli contained areas with elongated spermatids of normal morphology. In conclusion, the present data indicate that the impairment of spermatogenesis in HR6B knockout mice is caused by a defect in the germ cells and not in the testicular somatic cells.

PP – 033

CHARACTERIZATION OF RAT 100, A UBC4-DEPENDENT UBQUITIN-PROTEIN LIGASE INDUCED IN GERM CELLS OF THE RAT TESTIS AND SIMILAR TO THE DROSOPHILA HYPERPLASTIC DISCS GENE.

Ubiquitin (Ub) is conjugated to proteins by the sequential actions of three enzymes: Ub activating enzyme (E1) activates Ub and transfers it to a Ub conjugating enzyme (E2 or UBC) which interacts with a Ub-protein ligase (E3) to mediate ubiquitination of the target protein. We previously demonstrated activation of a specific E2 (UBC4) dependent pathway of conjugation during spermatogenesis. To further explore the role of this pathway, we are characterizing UBC4-dependent E3s expressed in the testis. Rat100 was previously identified by Muller et al. as a gene highly expressed in the testis. In vitro assays showed that Rat100 is an E3 that can accept Ub from various UBC4 isoforms. Western blotting showed that Rat100 protein was expressed at high levels in the testis. Expression was developmentally regulated in rat testis with peak expression in testes from 25 day old rats. In situ hybridization along with Western blotting of proteins from isolated germ cell populations revealed that Rat100 was germ cell specific with expression in pachytenic spermatocytes, round and elongating spermatids. Examination of Genbank sequences indicated that Rat100 sequence is similar to the Drosophila hyperplastic discs gene, in which mutations cause male sterility due to germ cell defects. Thus, specific induction during spermatogenesis of UBC4 and one of its interacting E3s, Rat100, likely leads to ubiquitination of specific proteins that must be destroyed for normal progression of this developmental process.

PP – 034

CYCLOSPORIN IMPROVES TESTIS GERM CELL SURVIVAL FOLLOWING MOUSE-TO-RAT TRANSPLANTATION.
Z Zhang, M Renfree, RV Short, Zoology Dept, University of Melbourne, Australia

Almost all recipient animals used in testis cell transplantation were either immunocompatible or immunodeficient since the first successful male germ cell transplantation was achieved in 1994. Many attempts, such as the cut of recipient’s spleen, donor-specific blood transfusion, and use of different immunosuppressive agents, were used to improve donor germ cell survival when immuno-incompatible rat was used as recipient in mouse to rat testis cell transplantation. Exogenous testosterone (T) or Lupron (GnRH agonist) was also used to stimulate spermatogenesis recovery after cytotoxic therapy and germ cell transplantation. We used the immunosuppressive agent cyclosporin A (CsA) plus T or Lupron following mouse germ cell transplantation into the testis of the rat. Germ cells were harvested from the cryptorchid testes of LacZ transgenic mice and transplanted via the rete testis to the testis of the recipient rats previously sterilized with busulfan. Results were assessed by histological examination and semi-quantitative analysis of X-gal staining 65-90 days after transplantation. Compared to the control group, CsA plus T or Lupron significantly improved mouse spermatogenesis in the rat’s testis. Our results suggest that a) immune rejection plays an important role in germ cell survival following inter-specific transplantation, b) CsA plus T or Lupron improves donor germ cell survival in the recipient testis and allows mouse germ cells to undergo complete spermatogenesis in the rat’s testis. 1. Ogawa, T., Dobrinski, I., Brinster, RL., 1999, Tissue & Cell 31:461-472 2. Meistrich, ML., Wilson, G., Huhtaniemi, I., 1999, Cancer Res. 59:3557-3560.
DNA REPLICATION AND GERM CELL DEATH DURING SPERMATOGENESIS IN THE RABBIT.
I Blanco-Rodriguez, Department of Cell Biology, School of Medicine, University of Valladolid, Spain.

Data on spermatogonium proliferation have led to hypothesis that the arrangement of stages along the length of the seminiferous tubules, or spermatogoniotic wave, might be based on the sequential synchronization of mitoses of committed spermatogonia (Franca et al., 1998; de Rooij and Grootegeest, 1998). The current knowledge on the extracellular and intracellular control of both cell proliferation and cell death at cell cycle checkpoints is consistent with this idea. Nevertheless, data on events subject or related to cell cycle checkpoints, such as DNA synthesis and cell death, are nearly restricted to small rodents. Here we analyze DNA synthesis as well as germ cell apoptosis in the rabbit. Our results contrast to the previous description of spermatogonium proliferation in this animal (Swierstra and Foote, 1963) and indicate that DNA replicates at stages similar to those in rats (Clermont, 1962) and mice (Monesi, 1962). Spontaneous apoptosis of both spermatogonia and spermatocytes is stage specific and mostly occurs coinciding with the mitotic peaks of the second and the third generation of spermatogonias, as it has been observed also in the rat testis (Blanco-Rodriguez, 1998).

EFFECTS OF HORMONES ON SPERMATOGENESIS IN MEN WITH HISTOLOGICAL DIAGNOSIS OF SPERMATOCYGENIC ARREST AT THE PRIMARY SPERMATOCYTE (PS) STAGE.
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We evaluated the effects of Profasi and Gonal-F (Serono Co.) on testicular function. Diagnostic testicular biopsy (DTB; hematoxylin-eosin stain) outcome and therapeutic testicular biopsy (TTB; mincing testicular tissue) outcome were compared before and after hormonal treatment in 2 groups of non-obstructive azoospermia (NOA) men. At the time of DTB intratesticular testosterone (ITT) was measured, whereas, at the time of TTB, fractions of dispersed cells were processed for transmission electron microscopy (TEM). Gonal-F (50 IU three times a week) and Profasi (2500 IU twice a week) were administered for 12 – 24 months in a) 27 men whose DTB showed arrest at the PS stage and TTB revealed absence of round spermatids (RSS) and sperms (group A) and b) 10 men whose DTB demonstrated arrest at the PS stage and the TTB was positive for RSS but negative for spermatozoa/elongated spermatids (group B). New DTB, TTB, and TEM techniques were performed at the end of the treatment. Observations of TTB samples post-treatment demonstrated few RSS or sperms in 9 men of group A. TEM showed elongating or elongated spermatids or sperms in the TTB materials of 8 men of group B post-treatment. Seven out of eight men of group B who responded to the treatment had low ITT profiles (less than 300ng/g testis) pre-treatment. Prior to the hormonal administration, the presence of RSS in the TTB of men with histological diagnosis of early maturation arrest or small ITT levels indicate good prognosis for response to the treatment.

SPTRX, A NOVEL THIOREDOXIN EXPRESSED DURING MAMMALIAN SPERM TAIL ELONGATION.

Thioredoxins (Trx) are small, ubiquitous redox proteins that function as general protein disulfide reductases and regulate different cellular mechanisms like DNA synthesis or apoptosis. We describe here the first member of the thioredoxin family in mammals with a tissue-specific distribution exclusively located in the testis (named Sptrx, for spermatid thioredoxin). Sptrx ORF encodes for a protein with two clear domains: an N-terminal domain with no homologue in the databases and a C-terminal domain typical of thioredoxins. Northern analysis and in situ hybridization showed that Sptrx mRNA is only expressed in testis, specifically in round and elongating spermatids. Immunostaining of human and rat testis sections using polyclonal antibodies identified the most prominent reactivity for Sptrx in the elongated spermatic tail with some reactivity remaining in the cytoplasmic droplet of ejaculated sperm. Sptrx appears to be a dimer in native conditions and is able to reduce insulin disulfide bonds in the presence of NADPH and thioredoxin reductase, the typical assay for thioredoxin enzymatic activity. Surprisingly, Sptrx is also able to oxidize diethiothreitol to disulfide in vitro. During mammalian spermiogenesis, extensive disulfide bonding is required to stabilize sperm structures like outer dense fibers (ODF) or fibrous sheath (FS). However, the mechanisms that regulate this process are not known. The identification of Sptrx with a dual reducing/oxidizing activity and its peak of expression at the spermatic stages when ODF and FS are formed suggest that it might be involved in regulating this essential step in spermiogenesis.
Abstracts – President’s Posters

PP - 039

THE MAJOR SUBACROSOMAL OCCUPANT OF BULL SPERMATOZOA IS A NOVEL HISTONE H2B VARIANT INVOLVED IN ACROSOMAL-NUCLEAR DOCKING DURING SPERMIOGENESIS.

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Recent studies on the structural composition of mammalian sperm heads have shown a congregate of unidentified proteins occupying the periphery of the mammalian sperm nucleus, forming a layer of condensed cytosol. These proteins are the perinuclear theca (PT) and can be categorized into SDS-soluble and SDS-insoluble components. The present study focused on identifying the major SDS-insoluble PT protein, which we localized to the subacrosomal layer of bovine spermatozoa and cloned by immunoscreening a bull testicular cDNA library. The isolated clones encode a protein of 122 amino acids that bears 67% similarity with histone H2B and contains a predicted histone fold motif. Hence, we identified this prominent subacrosomal component as a novel H2B variant, SubH2Bv. Computerized homology modeling of SubH2Bv has shown that its histone fold motif likely forms the same tertiary structure as H2B and that SubH2Bv contains cysteine residues in positions likely involved in dimer formation, explaining the SDS insolubility of the protein. The novel amino terminus of the protein contains a potential bipartite nuclear targeting sequence. Northern blot analyses of SubH2Bv mRNA expression, showed that it is testis specific and is also present in murid testes. Immunocytochemical analysis showed SubH2Bv intimately associates, temporally and spatially, with acrosome formation. While the molecular features of SubH2Bv are common to nuclear proteins, it is never seen developmentally within the nucleus of the spermatozoa. Considering its developmental and molecular characteristics, we have postulated roles of SubH2Bv in acrosome assembly and acrosome-nuclear docking.

PP - 040

LOCALIZATION OF A SPERM CD52 CARBOHYDRATE EPITOPE DURING EPIPDIYMAL MATURATION, CAPACITATION, AND THE ACROSOME REACTION.


The sperm glycoalyx represents an unexplored source of epitopes for immuncontraceptive development. S19, a monoclonal antibody that exhibits sperm-inhibitory activities, binds to an N-linked carbohydrate epitope on the GPI-anchored CD52 glycopeptide that is localized over the entire human sperm surface. Immunochemical and mass spectrometry analyses suggested that the S19 epitope is specific to a subset of CD52 glycoforms in the male reproductive tract. The S19 epitope was identified in the caput, corpus, and cauda epididymal epithelium and on epididymal spermatozoa. Variation in S19 immunoreactive bands on epididymal immunoblots suggested differential CD52 glycosylation along the length of the epididymis. The S19 epitope persists on spermatozoa capacitated in vitro as demonstrated by immunofluorescence. Following the acrosome reaction, S19 immunoreactivity indicative of sperm-surface CD52 was identified over all sperm domains including the anterior head region. Persistence of S19 immunoreactivity in this region following loss of the plasmalemma overlying the acrosome implicates a redistribution of CD52 into this area. Interestingly, potential species specificity of the S19 epitope was indicated by identification of the epitope in the human and chimpanzee but not in other primate species studied. Overall, sperm-inhibitory effects, tissue specificity, and persistence on all sperm-surface domains indicate the potential of the S19 carbohydrate epitope as an immunoccontraceptive vaccinogen.

PP - 041

POSSIBLE IN VIVO MODULATION OF HUMAN SPERM ACROSOME REACTION BY A CBG-LIKE PROTEIN

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The acrosome reaction (AR), a modified exocytotic process, is prerequisite for successful mammalian fertilisation. Enzymes thereby released are thought to play a role in the penetration of spermatozoa through the outer oocyte investments.

Our recent findings have shown that hFF (human follicular fluid) from which proteins and/or steroids (pro tease-, DCC treatment) had been removed could not induce the AR. However, after removal of steroids, the AR-inducing activity of hFF could be restored by exogenous progesterone, but only in the presence of intact protein.

In gel filtration experiments with H-progesterone-labelled hFF elution of the radioactivity signal in the high-molecular weight range, corresponding to bound progesterone, was found. The protein has been found to be immunologically identical with the corticosteroid-binding globulin (CBG), which has already been described and serves as a transport protein for progesterone and cortisol in the plasma. Our findings also show that the AR can be induced by CBG-progesterone complex at nanomolar concentrations, where both progesterone and the protein alone are unable to bring about an effect at these concentrations. In the culture medium of human cumulus oophorus cells, ARIS was detected by its biological activity as well as in Western blot analysis. Moreover, the secretion of a CBG-like protein was found in endothelial cells of the tubulus depending on the hormonal cycle.

Both immunological and radiochemical investigations strongly indicate that human cumulus cells actively express and secrete a CBG-like progesterone-binding protein.

PP - 042

MAP KINASES IN CYNONOMUS MONKEY SPERM HYPERACTIVATION. EN Haynes, RJ Swanson*, PF Blackmore*, and MC Mahony. Dept. of Biol. Sciences, Old Dominion Univ. and Dept. of OB/GYN and Phys. Sciences, Eastern VA Medical School, Norfolk, VA.

Capacitation related hyperactivated motility (HA) in macaque (Macaca fascicularis) sperm is dependent in vitro upon exogenous cyclic nucleotide mediators, caffeine and dbcAMP. We previously reported that protein tyrosine phosphorylation (TyrP) is an integral component of caffeine and cAMP stimulated HA. In this study, we investigate the involvement of mitogen-activated protein kinase (MAPK) signaling in macaque sperm HA. Method: Semen was collected from proven breeders via electroejaculation. After washing, sperm were incubated in the presence and absence of the MAPK kinase (MEK) inhibitor, PD 98059 for 2h at RT. Sperm were incubated with and without caffeine (1mM) and dbcAMP (1mM) for 0.5h at 37°C and 5% CO2. HA was determined by CASA (HTM-IVOS) using our established sorting criteria. TyrP of sperm tail proteins was determined by immunocytochemistry (ICC) with PY-20 antibody and immunoblotting examined TyrP of total proteins. Inhibition of MEK was determined by ICC of sperm exhibiting phosphorylated ERK 1/2 immunoreactivity.

Results: Treatment of sperm with PD 98059 resulted in a dose dependent decrease in phosphorylated ERK 1/2 from 60% (0µM) to 15% (baseline) (IC50=1µM). PY-20 immunoreactivity and motion characteristics (VCL, ALH, %HA) similarly decreased (IC50=3µM). However, complete inhibition of TyrP by PD-98059 of either sperm tail proteins by ICC or 55, 80, and 95 kDa proteins by immunoblotting was not observed at the doses tested. Conclusion: These results suggest MAP kinase is one, but not the exclusive upstream component in the signaling cascade of caffeine and cAMP stimulated HA motility in macaque sperm. (Supported by the Jeffress Memorial Trust.)
Abstracts – President's Posters

PP – 043

SEMENOGELIN, THE MAIN PROTEIN OF HUMAN SEMEN COAGULUM, INHIBITS SPERM CAPACITATION AND INTERFERS WITH THE SUPEROXIDE ANION GENERATED DURING THIS PROCESS. E de Lamirande*, L Yoshida*, M Yoshiike*, T Iwamoto* and C Gagnon*, Urology Research Lab, McGill Univ, Montréal, Québec, Canada and Dept of Urology, St Marianna Univ School of Medicine, Kawasaki, Japan.

Semenogelin (Sg), the major protein of the semen coagulum, is present at high concentrations in seminal vesicle secretions. We determined the effect of Sg on capacitation (CAP) which is the series of transformations that spermatozoa must undergo to become fertile. At 0.1-1.0 mg/ml (600-20-fold lower than semen concentration), Sg did not affect sperm motility (%) but totally prevented CAP induced by fetal cord serum ultraltrate; a partial inhibition of CAP was noted with 0.03 mg Sg/ml. There was also a dose-dependent decrease in the tyrosine phosphorylation of fibrous sheath proteins and O2*-related chemiluminescence. Rabbit seminal fluid (RNase), which has a high isoelectric point (pl=9.7) as Sg (pl=9.5), also prevented CAP and O2*-related chemiluminescence but to a lower extent, suggesting that Sg action on spermatozoa could be related to its positive charge at physiological pH. Sg, at 1, but not 0.3 or 0.1 mg/ml, scavenged the O2* generated by xanthine + xanthine oxidase and modified the kinetics of the reaction; RNase did not have such effects. Therefore, Sg is a potential scavenger for O2* but probably also affects the sperm oxidase. A high proportion of Sg is degraded by spermatozoa during the first 15 min of incubation. The resulting polypeptide patterns were reminiscent of those obtained with the prostate specific antigen as a proteolytic enzyme. Sg and/or its degradation products may be natural regulators of sperm function that prevent premature CAP. One mechanism of Sg action is by reducing O2* during incubation with CAP inducer.

Supported by the MRC of Canada and a Grant-in-Aid for Scientific Research from Education, Science, Sports and Culture of Japan.

PP – 044

INHIBITION OF SPERM-OOLEMMA INTERACTIONS BY THE EXTRACELLULAR DOMAINS OF CD9 AND CD81. CH Wong*, A Higginbotham*, P Monh*, LJ Partridge*, HD Moore1,2,*, 1Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK. 2Department of Obstetrics and Gynaecology, Jessop Hospital, Sheffield, S3 7RL, UK.

Tetraspanin molecules have been implicated in a range of cell adhesion and fusion processes including fertilization. Here, we have detected tetraspanins CD9 and CD81 (but not CD63) on the surface of hamster eggs by immunofluorescent localisation. The role of these tetraspanins during fertilization was investigated using recombinant proteins consisting of glutathione-S-transferase and the large extracellular domain of CD9 (GST-CD9), CD81 (GST-CD81) and CD63 (GST-CD63). GST-CD9 and GST-CD81 (not GST-CD63) bound specifically to oocytes and inhibited significantly sperm-egg binding and fusion during homologous hamster fertilization in vitro. In addition, anti-CD9 antibody and GST-CD9 inhibited human sperm penetration of zona-free hamster oocytes in vitro (hamster egg penetration test). GST-CD81 reduced human sperm binding to hamster oocytes but failed to inhibit sperm-egg fusion although it promoted strong aggregation of hamster oocytes. These results indicate that CD9 and CD81 may be important in modulating the activity of oocyte membrane proteins involved in hamster and human gamete adhesion and fusion processes.

PP – 045

SPERM-ZONA PELLUCIDA INTERACTION INVOLVES A CARBONYL REDUCTASE ACTIVITY IN HAMSTER. L Montfort*, G Frenette* and R Sullivan, Centre de recherche en biologie de la reproduction, Laval Universtiy

For successful fertilization to occur, spermatozoa must transit through an egg-specific extracellular matrix (zona pellucida) to reach and fuse with the oocyte plasma membrane. This process implies a mechanism of ligand-receptor recognition between zona pellucida (zp) and the acrosomal cap of sperm. Among sperm receptors, hamster sperm protein (P26h), acquired during epididymal transit, is proposed to be involved in sperm-egg binding. The cloning and characterization of the full length cDNA encoding hamster P26h revealed a 85% identity with porcine carbonyl reductase. To better understand mechanism by which P36h interacts with zona pellucida proteins, we investigated the involvement of carbonyl reductase activity in gamete interaction. In the present study, we show that inhibition of carbonyl reductase activity by diethylamino and phenylbutazone decreases sperm-zona pellucida binding without affecting motility, progressivity and acrosome integrity of sperm. We also detected and purified a NADPH oxidase activity from cauda epididymal spermatozoa which is specific of carbonyl reductase activities. Moreover, this enriched activity is related to an enriched fraction of P26h. Preliminary results on human also indicate an inhibition of sperm-zona pellucida binding by diethylamino and a carbonyl reductase activity of recombinant P34H, the human ortholog of P26h. Thus, inhibition of sperm-zp binding by carbonyl reductase inhibitors and active P26h in mature sperm bring about new elements on possible mechanisms involved in fertilization process. (Supported by MRC-Canada).

PP – 046

CHIMERIC CONSTRUCTS AND DOMINANT NEGATIVE COMPETITORS DEMONSTRATE THAT A FUNCTIONAL DOMAIN AND THE C-TERMINUS OF PROSAPOSIN (PSAP) ARE REQUIRED FOR ITS TRANSPORT TO THE LYOSOMES. S. Lefrançois, C. Knight*, D. Ham* and C.R. Morales Department of Anatomy and Cell Biology, McGill University, Montréal, Québec, Canada.

PSAP is a testicular glycoprotein that is either secreted or targeted to the lysosomes. Both forms of this protein are products of the same gene. PSAP is composed of four functional domains termed saposins A, B, C and D along with a highly conserved COOH region. Previous work has demonstrated that the COOH region is necessary but not sufficient to target prosaposin to the lysosomal compartment and the D domain was required for the targeting of this protein to the lysosomes. In this study, we examine the role of the COOH region and the functional domains A, B, C or D of PSAP by linking them to the secretary protein albumin. The targeting of these chimeric constructs to the lysosomes of COS-7 cells was determined by EM immunogold labelling. Our results indicate that domains A and D are capable of targeting PSAP to the lysosomal compartment. Domains B and C also target PSAP to the lysosomes but with a lower efficiency. A concern of this study, involving the truncated forms of PSAP attached to albumin, was whether or not these chimeric constructs utilized the same transport system as endogenous PSAP. Hence COS-7 cells were transfected with a D-COOH construct and used as a dominant negative competitor. Our results suggest that the dominant negative protein interfered with the targeting of endogenous PSAP. In conclusion, our results demonstrate that the A and D domains of PSAP are involved in its targeting to the lysosomes and to a lesser extent, the B and C regions as well. (Supported by CIHR).
Abstracts – President’s Posters

PP – 047
OVEREXPRESSION OF THE HUMAN Asp567Gly FSH RECEPTOR IN TRANSGENIC MICE.
M Simoni*, J Gromoll*, V Nordhoff*, S Schlatt*, L Poppiani*, E Nieschlag, Institute of Reproductive Medicine of the University, Muenster, Germany
In order to analyse FSH action on spermogenesis we generated transgenic mice over-expressing the human FSH receptor (FSHR) bearing the activating mutation Asp567Gly. Transgenic mice were generated by intranuclear injection of DNA constructs in which 1.5 kb of the human or mouse FSHR promoter was driving a human FSHR minigene carrying the Asp567Gly mutation. We obtained two founders bearing the human FSHR promoter construct and one bearing the mouse FSHR promoter construct. Transgenic mice were vital and fertile. Groups of F1 mice were killed at the age of 7, 14, 21, 35 and 56 days and organs collected. RT-PCR analysis showed that the transgene was expressed specifically in the testis. Testicular weight and serum testosterone were not different between wild type and transgenic mice at any time points. Testicular histology was normal. Northern blotting analysis showed that the FSHR expression in the testis was much higher in transgenic compared to non-transgenic littermates and that the 1.5 kb 5’ flanking region of the human FSHR drives the in vivo expression of the transgene in the testis and in the brain. By in situ hybridisation, the transgene was expressed in Sertoli cells and in germ cells. These data show that transgenic mice over-expressing a constitutively activated FSHR in the testis are fertile, without alterations in the testicular development. However, germ cells up to spermatocytes and round spermatids express the transgene as well. These data demonstrate that 1.5 kb of the human or mouse FSHR promoter are not sufficient to direct the expression of the FSHR only in Sertoli cells and that the overexpression of the transgene in the testis is compatible with normal fertility.

PP – 048
FUNCTIONAL DEVELOPMENT OF THE MARMOSET TESTIS; RELEVANCE TO PROTECTION OF SPERMATOCYTES IN CHILDREN TREATED FOR CANCER.
RM Sharpe, C Kelner*, K Morris, A Waring*, M Walker* H Fraser, P Saunders, C McKinnell*, MRC Human Reproductive Sciences Unit, 37 Chalmers Street, Edinburgh, Scotland, UK
Testis cell development was studied in marmosets from birth to adulthood to establish timing of functional development using immunohistochemical detection of protein markers. Where significant changes occurred in ‘infancy’, we established if GnRH antagonist (GnRHa) treatment prevented these changes. Maturational/functional status of Sertoli cells (SC) used sulphated glycoprotein-2 (SCGP-2), inhibin-α, AMH and androgen receptor (AR). Additionally, numbers of Leydig [3B-HSD-positive] cells were determined plus germ cell numbers/proliferation (BrdU, PCNA). Each marker showed a specific developmental pattern. For example, SCGP-2 was non-detectable in SC from 1 day to 20 weeks but switched on in ‘childhood’ (35 weeks) and increased further in intensity at puberty (60 weeks) and adulthood. SCGP-2 expression paralleled increase in GC volume/testis and lumen formation and volume. At 35 weeks, GnRHa-treated marmosets showed near complete reduction in SCGP-2, GC volume and lumen formation. Inhibin-α showed a different pattern to SCGP-2, as did AR expression in SC, which was minimal neonatally and developed in infancy and thereafter increased in intensity at puberty. LC were evident in neonates, decreased by 20 weeks, increased again at 35 weeks to 10% of adult LC volume/testis, a change inhibited by GnRHa; a large increase in LC volume occurred at puberty. Therefore, qualitative and semi-quantitative assessment of functional markers of testis cell maturation revealed specific age-related developmental patterns. Comparative studies of the human testis are in progress and these and the present studies will aid in design of strategies for testis protection from cytotoxic chemotherapy in children.

PP – 049
FOG-1 AND FOG-2: TESTICULAR EXPRESSION AND EFFECT ON GATA-MEDIATED GENE TRANSCRIPTION.
JJ Tremblay, NM Robert*, and RS Viger, CHUL Research Centre, Laval University, Ste-Foy, PQ, Canada.
The GATA transcription factors (G1 to G6) are crucial regulators of cell-specific gene expression. Three GATA factors are expressed in the testis. G4 and G1 are both present in Sertoli cells (SC) but at different stages: G4 marks fetal and neonate SC whereas G1 is present in postpubertal SC. G4 is also expressed in fetal and postnatal Leydig cells (LC). G6 is found specifically in germ cells (GC). Several gonadal target genes have been identified for GATA factors such as the hormone-encoding genes Mullerian inhibiting substance (MIS) and inhibit α, genes for transcription factors (TFS); steroidogenic factor 1 (SF-1) and Oct-4, and genes involved in steroidogenesis: P450 aromatase and steroidogenic acute regulatory protein (StAR). GATA factors mediate cell-specific gonadal gene expression through cooperative interactions with other TFS such as SF-1 on the MIS, inhibit α, and aromatase promoters, and with the bZIP TF C/EBPβ on the StAR promoter. GATA transcriptional activation is also modulated by two cofactors called Friend-of-GATA (FOG): FOG-1 for G1/2/3 and FOG-2 for G4/5/6. The expression of the FOG proteins and their effects on GATA-dependent transcription in the testis have not yet been assessed. We have used RT-PCR and immunohistochemistry to determine FOG expression in the testis. FOG-1 was specifically expressed in postpubertal SC whereas FOG-2 expression correlated with that of G4 and G6 and was found in GC, LC and weakly in SC. Transfection assays revealed that FOG-2 is a potent repressor of GATA-mediated transactivation and of GATA/SF-1 and GATA/C/EBPβ synergisms on a number of testicular target genes. Thus, our results support an important role for the FOG proteins in GATA-dependent gene transcription in multiple cell types of the testis.

PP – 050
ELEVATED 17α HYDROXYPROGESTERONE LEVELS IN INFERTILE PATIENTS WITH VARIOCOELE.
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The main goal of this study was to assess 17α Hydroxyprogesterone (17αHP) in Progesterone (P), 17,20 Desmolase (17,20D) and 17α Hydroxyprogesterone (C) serum levels in 23 patients with varicoceles (VP) (II and III) in order to eliminate any hormonal role in this pathology. In all cases Testosterone, LH and FSH were within normal ranges. In semen samples: 3 were normospermic, 18 were oligospermic and 2 asthenospermic. In the patients with normal semen there were also normal hormonal values. 17αHP in VP were: 2.187 ± 1.46 ng/ml which was markedly elevated (p<0.05) when compared to normal patients: 1.20 ± 0.70mg/ml (n=20). Nonetheless, in regard to normal patients two groups in VP could be identified; 1) (n=10) with remarkably higher values: 3.24 ± 1.60 ng/ml (p<0.001) and b)2: (n=13) with fairly similar to control group: 1.34 ± 0.48 ng/ml (p<0.2). The P4 (0.21 ± 0.46 ng/ml) in the total VP group was not statistically different than normals. In three patients P4 and 17HP were elevated (2.1, 1.2 and 1.6 ng/ml and 7.8, 3.7 and 2.7 respectively). In the total VP group the C (146.9 ± 82.0 ng/ml) and 11 DDOC (1.70 ± 1.11 ng/ml) were not different (p>0.2) when compared to normals (n=20): (C: 162.5 ± 87.5 ng/ml and 11 DDOC: 2.25 ± 1.75 ng/ml). Lower levels of 11DDOC: 0.40, 0.35 and 0.31 ng/ml were found in three patients, two of them correlated to low C, 26.0 and 77.0 ng/ml and high levels of 17HP; 2.1 and 3.7 ng/ml respectively. The rest of the hormonal levels were normal in the remaining 8 patients of the VP with elevated 17HP. It could be drawn as a conclusion that VP with abnormal seminal parameters had elevated 17HP, with a subgroup within accompanied with low C and 11DDOC. These preliminary findings suggest the heterogeneity of the varicocele etiology and although the eventual hormonal involvement could not be stated, it suggests a possible adrenal compromise (non-classic variety of MSO 21 blockage?) and/or elevations of Testosterone precursors from testicles (testicular demasculinization?).
Index of Abstract Authors
Index of Abstract Authors

Behdjani, R. PP-007 Boissonneault, G. P5/6-011 Burdorf, A. P3/4-053
Behringer, R.R. P5/6-004 Bojanic, N. P1/2-081 Burnazyan, R. P5/6-134
Bellastella, A. P3/4-061 Bojanic, N. P1/2-100 Burnett, A.L. P5/6-124
Benahmed, M. P1/2-027 Bojanic, N. P1/2-131 Buser, A. P3/4-131
Benny, P. PP-017 Bojovic, D. P5/6-023 Buser, A. P3/4-133
Benoff, S. P1/2-099 Bojovic, D. P3/4-077 Bushby, L. P5/6-083
Benoff, S. P5/6-099 Bojovic, S. P3/4-077 Busso, D. P1/2-052
Beorlegui, N. P5/6-029 Bojovic, S. P3/4-078 Bustos-Obregon, E. P5/6-093
Berger, P. PP-027 Bokiniec, M. P5/6-144 Bustos-Obregon, E. P5/6-107
Berges, L. P1/2-142 Bollendorf, A. P1/2-066 Buyukalpelli, R. P1/2-108
Bergh, A. P1/2-125 Bomgardner, D. PP-014 Caballero, J. P3/4-112
Bergh, A. P1/2-126 Bonaccorsi, L. PP-021 Calafell, J.M. P5/6-041
Berman, N. P1/2-135 Bongini, A. PP-021 Calamera, J. P5/6-041
Bern, O. P3/4-111 Bontis, G. P3/4-056 Calandra, R. P3/4-041
Bernie, J.E. PP-022 Borges, Jr., E. P3/4-056 Calvas, P. P1/2-061
Berthold, G. P1/2-016 Borges, Jr., E. P5/6-145 Calvo, J.C. P1/2-061
Bertolesi, G. P1/2-091 Bormman, M.S. P1/2-103 Campbell, A.J. P1/2-105
Betella, A. P5/6-131 Bormman, M.S. P5/6-078 Campo, S. P5/6-042
Betella, A. P5/6-113 Borsos, A. P5/6-085 Campo, S. P5/6-068
Betencourt, R. P3/4-140 Botchan, A. P5/6-035 Canas, B. P1/2-048
Bhat, A. P5/6-109 Bou, F. P5/6-019 Cancellaro, V.A. P3/4-079
Bhatia, V. P5/6-110 Boucher, D. P5/6-021 Carani, C. P5/6-115
Bhatia, V. P5/6-116 Boufe, F. P3/4-094 Carabajal, M.E. P1/2-041
Bhatia, V. P5/6-116 Bougrioua, S. P1/2-027 Carbone, U. P5/6-101
Bhattacharyya, A.K. P1/2-134 Bourroulilou, G. P5/6-061 Cardoso, E. P1/2-109
Bhavsar, N.G. PP-016 Bouroufoul, S.E. P1/2-029 Cardoso, E. P3/4-096
Bhullar, B. PP-031 Bowden, M. P5/6-040 Carloni, V. P3/4-134
Bianchessi, I. P3/4-059 Brach, V. P1/2-140 Carlsson, L. P5/6-147
Bianchi, A. P1/2-117 Brannam, H. P1/2-086 Caron, N. P5/6-011
Bianco, M.A. P1/2-024 Brannian, I. P5/6-152 Carreau, S. P1/2-026
Biancotti, J. PP-006 Bravo-Gatica, C. P1/2-086 Carreau, S. P1/2-027
Biancotti, J. P3/4-097 Breed, W.G. P5/6-152 Castoeiras, J. P1/2-064
Bieth, E. PP-006 Breitenstein, M.I. P3/4-003 Catt, J. P1/2-088
Bilaspuri, G.S. P1/2-001 Bremner, W.J. P3/4-067 Cavaria, B. P5/6-037
Bilaspuri, G.S. P1/2-021 Bremner, W.J. PP-004 Centola, G.M. P5/6-099
Bilaspuri, G.S. P5/6-050 Bressis, I.A. P1/2-029 Cermeño-Vivas, J.R. P5/6-150
Bissignani, S. P5/6-051 Brewis, I.A. PP-021 Cerpolini, S. P5/6-150
Biston, P. PP-012 Brito, M. P1/2-040 Cetinie, S. P3/4-124
Bivalacqua, T.J. P1/2-105 Brock, G. P5/6-126 Cetinie, S. P3/4-124
Bizzardo, D. P5/6-100 Brown, D.I. P3/4-133 Chan, P.T.K. P1/2-102
Bjartell, A. P5/6-034 Brown, M. P3/4-126 Chang, A.G. P5/6-124
Blackmore, P.F. P3/4-128 Bruchovsy, N. PP-028 Chansky, H.C. P5/6-084
Blanco, A. PP-050 Bruchovsy, N. P3/4-131 Chaudhury, K. P5/6-124
Blanco, A.M. PP-028 Brush, M. P3/4-133 Check, J.H. P1/2-066
Blanco, A.M. PP-037 Brush, M. P3/4-063 Check, J.H. P1/2-083
Blanco, P. P1/2-020 Büchel, D. P3/4-137 Check, J.H. P1/2-084
Blanco-Rodríguez, I. PP-035 Buchuie, A.D. P1/2-028 Check, J.H. P3/4-103
Blau, J. P1/2-028 Buffone, M. P1/2-042 Check, M.L. P1/2-083
Blasce, P. PP-019 Bujan, L. P1/2-028 Check, M.L. P1/2-084
Bobseine, K. P1/2-104 Bujan, L. P1/2-116 Check, M.L. P3/4-103
Bobseine, K.L. P5/6-085 Bujan, L. P1/2-142 Cheines, H.E. P1/2-038
Bögyi, G. P3/4-093 Bujan, L. P5/6-057 Chemes, H.E. P3/4-054
Bohler, C. P3/4-011 Bujan, L. PP-066 Chemes, H.E. P5/6-060
Boillard, M. P3/4-011 Bujan, L.
Index of Abstract Authors

Chen, Z.  P3/4-014  Correa, J.R.  P3/4-114  Dell, A.  PP-040
Chenlo, P.H.  P1/2-076  Cosgrove, D.  P3/4-085  den Ouden, K.  P3/4-026
Cheon, J.  PP-026  Cosgrove, D.  P5/6-080  Denduchis, B.  P5/6-048
Chernogorovna, A.  P1/2-044  Cosgrove, D.I.  P3/4-070  Deng, X.N.  P1/2-039
Chevalier, S.  P3/4-127  Costantino, A.  PP-004  Dettin, L.  P5/6-005
Chew, B.H.  PS/6-143  Crabo, B.G.  P3/4-046  Devroey, P.  P3/4-101
Chiariini-Garcia, H.  P3/4-028  Crescioni, C.  P1/2-013  Diaz, E.S.  P5/6-048
Chiariini-Garcia, H.  PS/6-016  Crete, M.H.  P3/4-006  Diaz, G.  P5/6-074
Chiariini-Garcia, H.  PS/6-045  Creus, M.  P3/4-113  Diaz-Guitierrez, O.  P1/2-144
Chitaley, K.  PP-024  Creus, S.  P5/6-068  Diekmann, A.B.  PP-040
Choe, J.K.  P1/2-084  Cristina Magli, M.  P3/4-108  Dimitriadis, D.  P5/6-043
Choi, J.B.  PS/6-127  Crane, J.K.  P5/6-124  DiPietro, I.  P3/4-012
Choi, J.B.  PS/6-128  Croxatto, H.  P1/2-140  Dirami, G.  P5/6-005
Choi, W.  PS/6-053  Csapo, B.  P5/6-085  Dobracheva, A.D.  P5/6-063
Choi, Y.D.  PS/6-098  Cusanicu, P.S.  P1/2-052  Dohle, G.R.  P5/6-133
Choudhury, S.  P3/4-047  Curi, S.M.  P1/2-076  Dominko, T.  P1/2-053
Christoph, A.  P3/4-055  Cyr, D.G.  P1/2-003  Doncel, G.F.  P1/2-031
Chun Yu Lee, E. PP-029  Cyr, D.C.  P1/2-004  Dong, Q.  P3/4-121
Chung, M.H.  PS/6-092  D'Abrizio, P.  P5/6-103  Dorval, V.  P1/2-036
Ciechan, J.  P3/4-138  D'Abrizio, P.  P1/2-089  Dubé, C.  P1/2-025
Cigorraga, S.  P3/4-054  D'Azzo, A.  P5/6-001  Dubin, N.H.  P3/4-046
Cigorraga, S.  PS/6-042  D'Azzo, A.  P5/6-010  Ducot, B.  P5/6-097
Cigorraga, S.  PS/6-048  Da Ros, V.G.  P5/6-120  Ducot, B.  P5/6-137
Cigorraga, S.  PS/6-066  Dam, V.  P1/4-127  Dueñas, J.L.  P1/2-064
Cimmino, F.  PS/6-101  Dambier, J.E.  P1/2-125  Dunlop, M.E.  P1/2-054
Citino, S.B.  P1/2-132  Dambier, J.E.  P1/2-126  Durrant, B.  P1/2-079
Civalleri, S.  P1/2-044  Damidomopoulos, A.E.  PP-038  Durutovic, O.  P1/2-100
Clark, J.C.  P3/4-067  Daneau, I.  PP-007  DuTeaux, S.B.  P5/6-087
Clavert, A.  PS/6-097  Darbandi, R.  PP-022  Duty, S.M.  P3/4-014
Clevenger, B.  P1/2-135  Daudin, M.  P1/2-116  Dym, M.  P3/4-029
Clulow, J.  PP-005  Daudin, M.  P1/2-142  Dym, M.  P5/6-005
Cohen, D.J.  P1/2-052  Daudin, M.  PS/6-061  Easton, R.  PP-040
Colao, A.  PS/6-101  Daudin, M.  PP-006  Eckert, G.  P5/6-056
Colombo, F.  P3/4-089  de Carvalho, C.E.  PS/6-006  Ercodi, H.  P1/2-035
Colombo, F.  PS/6-111  de Franca, L.R.  PS/6-045  Ercodi, H.  PP-005
Colombo, J.R.  P1/2-122  de Gouveia Brazao, C.A.  PS/6-133  Eid, M.  P3/4-125
Colpi, G.M.  P3/4-108  de Kretser, D.M.  P1/2-137  Eid, N.A.S.  P5/6-091
Colpi, G.M.  PS/6-112  de Lamiarade, E.  P1/2-030  Eisenbach, M.  P1/2-043
Comini, E.  P3/4-096  de Lamiarade, E.  PP-043  Eisenbach, M.  P1/2-044
Comoz, F.  P1/2-098  De Marinis, L.  P1/2-115  El Guiziri, D.  P1/2-090
Concha, I.I.  P1/2-040  De Marinis, L.  P1/2-117  El Mulla, K.  P1/2-090
Conde, J.M.  PS/6-122  De Mas, P.  P5/6-061  El-Amrawi, G.  P1/2-061
Confalonieri, S.  P3/4-089  de Meio Rocha, D.C.  P5/6-071  El-Ashmawy, N.E.  P5/6-100
Contreras, L.N.  P1/2-109  de Miguel, M.P.  PP-002  El-Gammad, M.  P5/6-146
Conoray, A.I.  P3/4-012  De Muijnck Keizer, S.  P3/4-053  El-Ghahani, F.  PP-008
Conway, A.I.  PP-018  De Paulis, A.  P3/4-096  El-Ghobashy, A.A.  P1/2-082
Cooke, N.  P1/2-096  de Rooij, D.G.  P3/4-096  El-Kamshoushi, A.A.  P1/2-111
Cooper, D.  PS/6-139  De Rosa, M.  P3/4-026  El-Meleegy, A.  P5/6-073
Cooper, T.G.  PS/6-140  De Rosa, M.  P5/6-110  El-Nashar, A.  P1/2-107
Cordeschi, G.  P1/2-089  De Stefano, C.  P1/2-089  El-Sewfie, A.  P3/4-073
Cordeschi, G.  PS/6-001  Delbeljuk, L.  P5/6-071  El-Tonsy, M.H.  P5/6-027
Côrdoba, M.  PS/6-030  DeBellefeuille, S.  P1/2-004  Elakhras, A.I.  P5/6-108
Coronel, C.  P1/2-024  Del Rio, A.G.  P1/2-005  Elashmawy, N.E.  P5/6-076
Correa, J.R.  P1/2-101  Delci, T.G.  P1/2-068  Elhanbly, S.  P3/4-083
Index of Abstract Authors

Grigoryan, S.B.
Griswold, M.D.
Grizard, G.
Grobler, S.
Gromoll, J.
Groome, N.
Groome, N.P.
Grootegoed, J.A.
Grossman, G.
Grossman, J.
Grunewald, S.
Guschwitz, W.
Guragnia, C.
Guay, A.T.
Guay, S.
Guazzzini, B.
Guerif, F.
Guha, K.
Guillen, J.
Gutierrez, J.A.
Gutierrez, J.A.
Guzman, M.
Gvinepadze, N.
Gwathmey, T.M.
Habermann, H.
Habermann, W.
Haendler, B.
Haider, S.C.
Hair, W.M.
Hall, S.H.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Hallak, J.
Ham, D.
Ham, D.
Hamil, K.G.
Hamilton, D.W.
Hamilton, D.W.
Hammadeh, M.E.
Hammadeh, M.E.
Han, S.W.
Han, S.W.
Han, S.Y.
Handelsman, D.J.
Handelsman, D.J.
Handelsman, D.J.
Hansen, K.
Hansson, J.
Harayama, H.
Hardisson, D.
Hardisson, D.
Hardy, M.P.
Haren, M.T.

Hariu, K.
Harlamov, S.Y.
Hassan, H.A.
Hauser, R.
Hauser, R.
Hauser-Kronberger, C.
Haynes, E.N.
He, L.
Hegaz, A.
Hegaz, A.
Heicappell, R.
Helal, S.
Hellstrom, W.I.G.
Hellstrom, W.I.G.
Hellstrom, W.I.G.
Henkel, R.
Hermo, L.
Hermo, L.
Hermo, L.
Herr, J.C.
Hessian, P.
Heuermann, T.
Higginbottom, A.
Hill, S.T.
Hunting, A.
Hunting, A.
Hinton, B.T.
Hippach, M.
Hippach, M.
Hochereau de Reviers, M.T.
Hofmann, M-C.
Hofmann, M-C.
Holm, C.
Holm, C.
Holmes, P.V.
Holyoake, A.J.
Horak, S.
Horneck, J.R.
Horte, A.
Hou, R.
Houwing, N.S.
Houwing, N.S.
Howard, J.G.
Howard, J.G.
Howard, J.G.
Hoyos, D.
Hu, D.M.
Huang, H.F.S.
Huang, M.
Huang, S.
Huang, T.
Huang, W.
Huang, Y.
Huang, Y.
Huang, Y.F.
Huang, Y.F.

Huang, Y.F.
Huebler, D.
Huhtaniemi, I.
Hui, L.
Huikko, M.P.
Huk, J.
Huk, J.
Hurley, I.R.
Hurley, I.R.
Husainov, T.E.
Husainov, T.E.
Huyghe, E.
Huyghe, E.
Hyun, I.S.
Kim, I-S.
Iaconelli, Jr., A.
Ibrahim, N.M.
Ichikawa, T.
Idriss, D.
Igarashi, T.
Iglesias, I.
Ignzot, G.G.
Iguchi, N.
Iguchi, N.
Iizumi, T.
Illyperuma, I.
Imam, H.
Irsula, A.
Irvine, D.S.
Irvine, D.S.
Irvine, S.
Ishiyakawa, H.
Ismoldaev, E.S.
Ito, H.
Ito, Y.
Ivarsson, S.
Iwabuchi, M.
Iwabuchi, M.
Iwamoto, T.
Iwamoto, T.
Iwamoto, T.
Iwamoto, T.
Izadiyar, F.
Izbiasarov, A.I.
Izbiasarov, A.I.
Izopet, J.
Jackson, P.
Jacobson, J.L.
Jain, N.
Jain, S.K.
Jakiel, G.
Jakiel, G.
Jalali, S.
Jallous, H.
Janigava, M.
Jankowska, A.C.
Jarvi, K.
Jarvi, K.
## Index of Abstract Authors

<table>
<thead>
<tr>
<th>Author</th>
<th>Paper Details</th>
<th>Abstract Title</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvi, K.</td>
<td>P5/6-032</td>
<td>Kasyan, A.</td>
<td>P5/6-080</td>
</tr>
<tr>
<td>Jarvi, K.</td>
<td>P5/6-143</td>
<td>Kasyan, A.</td>
<td>P5/6-030</td>
</tr>
<tr>
<td>Jarvi, K.</td>
<td>P5/6-151</td>
<td>Katsiya, G.V.</td>
<td>P5/6-063</td>
</tr>
<tr>
<td>Jedzura, A.</td>
<td>P3/4-138</td>
<td>Katsoff, D.</td>
<td>P1/2-066</td>
</tr>
<tr>
<td>Jung, B.C.P.</td>
<td>P1/2-123</td>
<td>Kaufman, S.</td>
<td>P1/2-085</td>
</tr>
<tr>
<td>Jenkins, N.M.</td>
<td>P1/2-029</td>
<td>Kawamura, H.</td>
<td>P1/2-085</td>
</tr>
<tr>
<td>Jensen, L.</td>
<td>P1/2-115</td>
<td>Kelleher, S.</td>
<td>P3/4-012</td>
</tr>
<tr>
<td>Jensen, L.</td>
<td>P1/2-117</td>
<td>Kelnar, C.</td>
<td>P5/6-048</td>
</tr>
<tr>
<td>Jensen, T.K.</td>
<td>P5/6-142</td>
<td>Kempinas, W.D.</td>
<td>P1/2-009</td>
</tr>
<tr>
<td>Jorgensen, N.</td>
<td>P5/6-142</td>
<td>Kempinas, W.D.</td>
<td>P5/6-094</td>
</tr>
<tr>
<td>Jobson, S.</td>
<td>P1/2-054</td>
<td>Kermes, K.</td>
<td>P5/6-070</td>
</tr>
<tr>
<td>Jockenhoevel, F.</td>
<td>P3/4-055</td>
<td>Kersten, C.A.</td>
<td>P5/6-031</td>
</tr>
<tr>
<td>Joe, Y.</td>
<td>P3/4-060</td>
<td>Khalili, M.A.</td>
<td>P5/6-130</td>
</tr>
<tr>
<td>Johnson, E.W.</td>
<td>P5/6-005</td>
<td>Khamel, K.M.</td>
<td>P1/2-113</td>
</tr>
<tr>
<td>Jones, R.C.</td>
<td>P1/2-033</td>
<td>Khoo, J.G.I.</td>
<td>P5/6-037</td>
</tr>
<tr>
<td>Jones, R.C.</td>
<td>P1/2-035</td>
<td>Ki, H.Y.</td>
<td>P5/6-092</td>
</tr>
<tr>
<td>Jorgensen, N.</td>
<td>PP-005</td>
<td>Kiefer, D.</td>
<td>P1/2-084</td>
</tr>
<tr>
<td>Jost, L.</td>
<td>P5/6-069</td>
<td>Kiefer, D.</td>
<td>P5/6-092</td>
</tr>
<tr>
<td>Jounnutt, P.</td>
<td>P1/2-086</td>
<td>Kim, B.H.</td>
<td>P5/6-127</td>
</tr>
<tr>
<td>Jounnutt, P.</td>
<td>P1/2-072</td>
<td>Kim, H.S.</td>
<td>P5/6-128</td>
</tr>
<tr>
<td>Joubert, G.</td>
<td>P5/6-010</td>
<td>Kim, I.Y.</td>
<td>PP-026</td>
</tr>
<tr>
<td>Junco, A.</td>
<td>P5/6-086</td>
<td>Kim, J.J.</td>
<td>P5/6-104</td>
</tr>
<tr>
<td>Jungwirth, A.</td>
<td>P1/2-062</td>
<td>Kim, J.J.</td>
<td>P5/6-125</td>
</tr>
<tr>
<td>Jungwirth, A.</td>
<td>P5/6-119</td>
<td>Kim, J-J.</td>
<td>KS-004</td>
</tr>
<tr>
<td>Juttmann, R.</td>
<td>P3/4-053</td>
<td>Kim, S.C.</td>
<td>KS-005</td>
</tr>
<tr>
<td>Kabbaj, O.</td>
<td>P3/4-044</td>
<td>Kim, S.W.</td>
<td>KS-006</td>
</tr>
<tr>
<td>Kabbaj, O.</td>
<td>P3/4-050</td>
<td>Kim, T.S.</td>
<td>KS-007</td>
</tr>
<tr>
<td>Kadowitz, P.J.</td>
<td>P3/4-087</td>
<td>Kim, T.S.Y.</td>
<td>KS-008</td>
</tr>
<tr>
<td>Kajdaniuk, D.</td>
<td>P3/4-058</td>
<td>Kim, Y.S.</td>
<td>K5/6-028</td>
</tr>
<tr>
<td>Kajdaniuk, D.</td>
<td>P5/6-067</td>
<td>Kim, Y.S.</td>
<td>K5/6-104</td>
</tr>
<tr>
<td>Kamal, A.</td>
<td>P3/4-104</td>
<td>Kim, Y.S.</td>
<td>K5/6-127</td>
</tr>
<tr>
<td>Kamal, K.</td>
<td>P5/6-143</td>
<td>Kim, Y.W.</td>
<td>K5/6-128</td>
</tr>
<tr>
<td>Kamal, K.M.</td>
<td>P5/6-022</td>
<td>King, S.A.</td>
<td>P3/4-036</td>
</tr>
<tr>
<td>Kamal, K.M.</td>
<td>P5/6-031</td>
<td>Kirby, J.L.</td>
<td>KS-019</td>
</tr>
<tr>
<td>Kamal, K.M.</td>
<td>P5/6-032</td>
<td>Kishita, O.</td>
<td>KS-040</td>
</tr>
<tr>
<td>Kamal, K.M.</td>
<td>P5/6-151</td>
<td>Kishita, O.</td>
<td>KS-041</td>
</tr>
<tr>
<td>Kamarieh, M.</td>
<td>P3/4-068</td>
<td>Kitahara, S.</td>
<td>KS-042</td>
</tr>
<tr>
<td>Kamischke, A.</td>
<td>PP-003</td>
<td>Kleiman, S.</td>
<td>P3/4-035</td>
</tr>
<tr>
<td>Kan, F.W.K.</td>
<td>P3/4-002</td>
<td>Klinefelter, G.R.</td>
<td>P1/2-104</td>
</tr>
<tr>
<td>Kanakas, N.</td>
<td>P5/6-114</td>
<td>Klinefelter, G.R.</td>
<td>P3/4-022</td>
</tr>
<tr>
<td>Kanakas, N.</td>
<td>P3/4-036</td>
<td>Klinefelter, G.R.</td>
<td>P5/6-046</td>
</tr>
<tr>
<td>Kandil, S.H.</td>
<td>P3/4-062</td>
<td>Klinefelter, G.R.</td>
<td>P5/6-153</td>
</tr>
<tr>
<td>Kandirali, E.</td>
<td>P5/6-027</td>
<td>Klinefelter, G.R.</td>
<td>PP-019</td>
</tr>
<tr>
<td>Kaneo, S.</td>
<td>P1/2-063</td>
<td>Kloz, K.L.</td>
<td>PP-040</td>
</tr>
<tr>
<td>Kaneo, S.</td>
<td>P1/2-075</td>
<td>Knaak, C.</td>
<td>P1/2-019</td>
</tr>
<tr>
<td>Kang, I.H.</td>
<td>P5/6-092</td>
<td>Knee, R.A.</td>
<td>PP-005</td>
</tr>
<tr>
<td>Kang-Shou, Y.</td>
<td>P3/4-007</td>
<td>Knight, C.</td>
<td>PP-046</td>
</tr>
<tr>
<td>Kanwar, U.</td>
<td>P1/2-045</td>
<td>Kobayashi, H.</td>
<td>P5/6-024</td>
</tr>
<tr>
<td>Kapolla, N.</td>
<td>P5/6-044</td>
<td>Kobayashi, T.</td>
<td>P3/4-060</td>
</tr>
<tr>
<td>Karbsbrum, S.</td>
<td>P3/4-057</td>
<td>Koga, M.</td>
<td>P3/4-034</td>
</tr>
<tr>
<td>Karsza, A.</td>
<td>P3/4-074</td>
<td>Koh, E.</td>
<td>P1/2-092</td>
</tr>
<tr>
<td>Kaskar, K.</td>
<td>P1/2-101</td>
<td>Koh, S.K.</td>
<td>PP-026</td>
</tr>
<tr>
<td>Kaskar, K.</td>
<td>P3/4-065</td>
<td>Köhn, F.M.</td>
<td>P3/4-023</td>
</tr>
<tr>
<td>Kaskar, K.</td>
<td>P3/4-114</td>
<td>Köhn, F.M.</td>
<td>P5/6-039</td>
</tr>
<tr>
<td>Kassai, Z.</td>
<td>P5/6-085</td>
<td>Kohroki, J.</td>
<td>P3/4-034</td>
</tr>
<tr>
<td>Kasyan, A.</td>
<td>P5/6-072</td>
<td>Kolesnikova, G.S.</td>
<td>P5/6-063</td>
</tr>
<tr>
<td>Author</td>
<td>Page/Section</td>
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<tr>
<td>------------------------</td>
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<tr>
<td>P5/6-054</td>
<td>Lóivukene, K.</td>
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<tr>
<td>P5/6-082</td>
<td>Lokshin, V.N.</td>
<td></td>
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<tr>
<td>P5/6-137</td>
<td>Lombardi, G.</td>
<td></td>
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</tr>
<tr>
<td>P3/4-064</td>
<td>López, A.</td>
<td></td>
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<tr>
<td>P1/2-032</td>
<td>López, J.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-036</td>
<td>Lopez, M.C.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-055</td>
<td>Lopez, M.C.</td>
<td></td>
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</tr>
<tr>
<td>P3/4-017</td>
<td>Loseth, K.J.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-013</td>
<td>Low, B.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-132</td>
<td>Lowrey, F.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-092</td>
<td>Lu, J.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-124</td>
<td>Lu, J.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-098</td>
<td>Lu, N.G.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-125</td>
<td>Lu, N-Q.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-064</td>
<td>Lu, N-Q.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-098</td>
<td>Lucas, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-049</td>
<td>Lucon, A.M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-030</td>
<td>Lucon, A.M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-019</td>
<td>Lucon, A.M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP-046</td>
<td>Lucon, A.M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-012</td>
<td>Luconi, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-136</td>
<td>Luconi, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-112</td>
<td>Lue, Y.H.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-079</td>
<td>Lundwall, K.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-149</td>
<td>Lustig, L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-018</td>
<td>Lustig, L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-082</td>
<td>Lyer, K.S.N.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-057</td>
<td>Lysiak, J.J.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-098</td>
<td>Macerola, B.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-095</td>
<td>Madeo, B.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-085</td>
<td>Magasi, P.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-014</td>
<td>Maggi, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP-024</td>
<td>Maggi, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-079</td>
<td>Magyar, E.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-082</td>
<td>Mahale, S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-079</td>
<td>Mahoney, M.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-103</td>
<td>Mahran, A.M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-105</td>
<td>Majik-Singh, N.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-106</td>
<td>Major, L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-146</td>
<td>Malm, J.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-091</td>
<td>Malm, J.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-022</td>
<td>Mamaladze, V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-139</td>
<td>Mammi, C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-009</td>
<td>Mamoulakis, C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-129</td>
<td>Mancini, A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-086</td>
<td>Mancini, A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-054</td>
<td>Mancini, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-074</td>
<td>Mancini, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-012</td>
<td>Mändar, R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP-018</td>
<td>Manicardi, G.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP-013</td>
<td>Manicardi, G.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-099</td>
<td>Manjunath, P.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP-038</td>
<td>Manjunath, P.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-067</td>
<td>Mansi, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-091</td>
<td>Mansour, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-083</td>
<td>Mansour, R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-086</td>
<td>Mantovani, F.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-070</td>
<td>Mantovani, F.</td>
<td></td>
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</tr>
<tr>
<td>P5/6-135</td>
<td>Mantzavinos, T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-110</td>
<td>Manujath, P.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-101</td>
<td>Mapletonf, R.J.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-064</td>
<td>Marandola, P.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-073</td>
<td>Marchlewksa, K.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-001</td>
<td>Marcos, M.S.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-032</td>
<td>Mardomingo, C.T.</td>
<td></td>
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</tr>
<tr>
<td>P3/4-046</td>
<td>Marek, B.</td>
<td></td>
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</tr>
<tr>
<td>P3/4-067</td>
<td>Marek, B.</td>
<td></td>
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<tr>
<td>P3/4-086</td>
<td>Marin, P.</td>
<td></td>
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<tr>
<td>P3/4-037</td>
<td>Marin, P.</td>
<td></td>
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<tr>
<td>P3/4-038</td>
<td>Marin-Briggler, C.</td>
<td></td>
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<tr>
<td>P3/4-090</td>
<td>Marin-Briggler, C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-090</td>
<td>Maris, E.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-087</td>
<td>Maris, F.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-097</td>
<td>Marmar, J.L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-067</td>
<td>Martic, M.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-119</td>
<td>Martín-DeLeon, P.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-122</td>
<td>Martínez de Osaba, M.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-123</td>
<td>Martínez, R.</td>
<td></td>
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<tr>
<td>P1/2-013</td>
<td>Marzuki, S.</td>
<td></td>
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</tr>
<tr>
<td>P5/6-149</td>
<td>Mate, K.E.</td>
<td></td>
<td></td>
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<tr>
<td>P5/6-018</td>
<td>Matsuda, T.</td>
<td></td>
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<tr>
<td>P3/4-123</td>
<td>Matsumiya, K.</td>
<td></td>
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<tr>
<td>P5/6-048</td>
<td>Matsumoto, A.M.</td>
<td></td>
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<tr>
<td>P5/6-049</td>
<td>Matsushita, T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-136</td>
<td>Matulevicius, V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-042</td>
<td>Matulevicius, V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-046</td>
<td>Matusik, R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-115</td>
<td>Matusik, R.J.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-074</td>
<td>Mayerhofer, A.</td>
<td></td>
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</tr>
<tr>
<td>P5/6-117</td>
<td>Mazurczak, T.</td>
<td></td>
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<tr>
<td>P1/2-112</td>
<td>Mazzoni, G.</td>
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</tr>
<tr>
<td>P1/2-136</td>
<td>McCauley, T.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP-042</td>
<td>McCullough, A.R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-036</td>
<td>McGill, U.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-081</td>
<td>McKinnell, C.</td>
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<tr>
<td>P3/4-075</td>
<td>McLachlan, R.I.</td>
<td></td>
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<tr>
<td>P5/6-058</td>
<td>Medeiros, A.R.C.</td>
<td></td>
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</tr>
<tr>
<td>PP-009</td>
<td>Medeiros, A.R.C.</td>
<td></td>
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<tr>
<td>P5/6-055</td>
<td>Medina, M.B.</td>
<td></td>
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<tr>
<td>P3/4-129</td>
<td>Meherji, P.K.</td>
<td></td>
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<tr>
<td>P5/6-043</td>
<td>Mehta, M.N.</td>
<td></td>
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<tr>
<td>P1/2-115</td>
<td>Meineke, V.</td>
<td></td>
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</tr>
<tr>
<td>P1/2-117</td>
<td>Meistrich, M.L.</td>
<td></td>
<td></td>
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<tr>
<td>P5/6-112</td>
<td>Melekos, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-117</td>
<td>Mellinger, U.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/2-070</td>
<td>Meltzer, S.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-100</td>
<td>Menard, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-034</td>
<td>Menard, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-006</td>
<td>Mencacci, C.</td>
<td></td>
<td></td>
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<tr>
<td>P3/4-024</td>
<td>Mendeluk, G.R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-073</td>
<td>Mendis-Handagama, S.M.L.C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3/4-095</td>
<td>Mensi, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5/6-067</td>
<td>Merhej, S.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Index of Abstract Authors

Merico, M.
Merico, M.
Meriggiola, M.C.
Meroni, S.
Meroni, S.
Meroni, S.B.
Mishref, A.
Messias, A.G.
Meucci, E.
Meucci, E.
Michalak, J.
Micic, S.
Micic, S.
Micic, S.
Mielnik, A.
Mieusset, R.
Mieusset, R.
Mieusset, R.
Millardi, D.
Millardi, D.
Millan, C.
Millan, C.
Miller, K.
Miller, M.G.
Mills, T.
Minelli, A.
Minucci, S.
Miranda, I.R.
Mirfin, K.
Mishra, R.K.
Miska, W.
Mitchell, A.
Mittelmann, L.
Miyagawa, I.
Miyaji, K.
Miyazaki, M.
Mizokami, A.
Moein, M.
Moeloek, N.
Moffatt, O.
Moffatt, O.
Mohamed, E.F.
Mohamed, M.S.
Mohapatra, B.
Molina, C.A.
Molina, S.
Moline, F.C.
Molla, S.
Mondaini, N.
Monga, M.
Monga, M.
Monga, M.
Monga, M.
Monga, M.
Monga, M.
Nada, E.A.
Nakaizumi, C.
Nakamura, Y.
Nakayama, T.
Nam, S.Y.
Namiki, M.
Nassar, S.O.
Nassar, S.O.
Nauc, V.
Naughton, C.K.
Nazaryan, V.K.
Nazaryan, V.K.
Nazian, S.I.
Negri, L.
Negri, L.
Negri, L.
Neis, O.
Nelson, A.
Nelson, D.R.
Nelson, D.R.
Nelson, D.R.
Nelson, D.R.
Nevess, E.S.
Nevess, E.S.
Newcombe, N.
Nicholson, H.D.
Nicholson, H.D.
Nicholson, H.D.
Niederberger, C.
Niederberger, C.
Niepoorniszke, H.
Nieschlag, E.
Nieschlag, E.
Nieschlag, E.
Niewelstein, P.F.E.M.
Nijman, R.
Nikopoulos, S.
Nilsson, O.
Nishida, T.
Nishimune, Y.
Nishimune, Y.
Nistal, M.
Nistal, M.
Nobori, T.
Noe, G.
Nojima, H.
Nonnenmacher, L.
Nordhoff, V.
Nordin, B.E.C.
Norton, E.J.
Noseir, W.M.B.
Notaro, A.
Novella, M.
Noya, C.
Nozaki, M.
Nozawa, S.
Nyquist, S.E.

212 | VIIth International Congress of Andrology
Index of Abstract Authors

O'Carroll, S. PP-017 Otero, P. P3/4-057 Pellizzari, E. P3/4-054
O'Connor, D.B. P1/2-056 Othman, I.A. P1/2-107 Pellizzari, E. P5/6-042
O'Donnell, L. P1/2-137 Otsubo, Y. P5/6-091 Pellizzari, E. P5/6-048
O'Flaherty, C. P5/6-029 Ottenweller, J.E. P3/4-033 Pellizzari, E. P5/6-066
O'Grady, M.J. P5/6-082 Oughred, R. PP-033 Penfold, L. P1/2-132
O'Rand, M.G. PP-013 Ozcinar, E. P3/4-124 Penna Videa, S.J. P5/6-150
O'Sullivan, J. P3/4-131 Ozcinar, E. Perco, M. PP-050
O'Sullivan, J. P5/6-083 Ozu, M. P5/6-049 Perreault, S.D. P1/2-104
Obinata, M. PP-012 Paasch, U. P1/2-037 Petrusz, P. PP-013
Oettel, M. P3/4-055 Packman, K. P3/4-132 Pfister, G. P3/4-130
Ogura, K. P1/2-121 Packman, K. PP-029 Phang, D. P5/6-022
Oh' hara, I. P3/4-031 Padrón-Medina, A. P5/6-150 Phang, D. P5/6-031
Ohta, S. P1/2-069 Paesano, L. P5/6-101 Pia Ferraretti, A. P3/4-108
Ohta, S. P3/4-034 Paesano, L. P3/4-071 Piediferro, G. P5/6-112
Ojoo, R.O. P1/2-050 Pagnon, V. P5/6-123 Pierik, F.H. P3/4-053
Okar, I. P3/4-124 Pagnon, V. P5/6-064 Pierik, F.H. P5/6-133
Okar, I. P5/6-052 Paick, J-S. P3/4-143 Pietere, J. P5/6-086
Okezie, N. P3/4-133 Palacios, J. P1/2-005 Pignatario, O. P5/6-041
Okol, R. P3/4-004 Palladino, M.A. P5/6-059 Pilder, S.H. P1/2-051
Okol, R. PP-031 Palmer, J.D. P5/6-059 Pilon, N. PP-007
Okol, R.J. PP-039 Palti, Z. P1/2-093 Pinto, C. P5/6-107
Oko, I. PP-030 Pandolfi, C. P1/2-046 Pintos, L. P5/6-030
Okubo, K. P3/4-133 Pancy, J.J. P5/6-054 Pisani, E. P3/4-089
Okulov, A.B. P1/2-005 Papadimas, J. P3/4-056 Pisani, E. P5/6-111
Okuyama, A. P3/4-034 Papp, G. P1/2-112 Pizzorno, M.C. P3/4-045
Olds-Clarke, P. P1/2-051 Papp, G. P3/4-074 Plante, P. P5/6-057
Oliva, A. P5/6-089 Papp, G. P3/4-075 Plessis, G. P1/2-098
Oliva, A. PP-020 Pappa, A. P5/6-044 Plotka, E. P1/2-132
Oliva, R. PP-039 Pappin, D. P1/2-048 Pogach, L.M. P3/4-033
Oliva, S.U. P5/6-094 Park, H.S. P5/6-104 Ponchietti, R. PP-021
Oliver, S.A. P5/6-020 Park, H.S. P5/6-127 Pontonnier, F. P1/2-116
Olivera-Angel, M. P1/2-073 Park, H.S. P5/6-128 Pontonnier, F. P5/6-057
Ollero, M. P3/4-001 Park, H.S. PP-026 Pontonnier, F. PP-006
Ollero, M. P3/4-032 Park, K.L. P5/6-092 Porrut, D. P3/4-059
Ollero, M. P5/6-027 Parshad, R.K. P5/6-015 Poveda, J.D. P1/2-141
Ollero, M. PP-037 Partridge, L.J. PP-044 Powell, J. P3/4-041
Olmedo, S.B. P1/2-038 Pasquali, D. P3/4-061 Pozzoni, F. P5/6-111
Olsen, I. P5/6-141 Pasqualto, FF. P1/2-067 Predari, S. P3/4-096
Olsson, A.M. P3/4-123 Pasqualto, FF. P1/2-119 Preiks, R.T. P3/4-049
Ong, D. P1/2-007 Pasqualto, FF. P1/2-122 Prindantseva, T.P. P3/4-051
Ooms, M.P. PP-032 Pasqualto, FF. P1/2-123 Prins, G.S. P5/6-120
Orgebin-Crist, M-C. P1/2-007 Pasqualto, FF. P3/4-091 Properzi, G. P5/6-001
Orgebin-Crist, M-C. P1/2-018 Pasqualto, FF. P3/4-102 Pruysier, E. P1/2-137
Orgebin-Crist, M-C. PP-012 Pasqualto, FF. P5/6-145 Przytula -Pilat, M. P5/6-144
Orlando, G. P3/4-059 Pasquier, C. P1/2-142 Publicover, S.J. P1/2-034
Oryan, S. P3/4-048 Patelli, E. P3/4-089 Puel, J. P1/2-142
Oschy, I. P1/2-085 Patelli, E. P5/6-111 Puerto, B. P3/4-113
Oshio, S. P1/2-077 Patrizio, P. P3/4-001 Pugliese, M.N. P1/2-076
Oshio, S. P1/2-078 Pauls, K.P. P1/2-042 Pujianto, D.A. P1/2-133
Osman, I. P1/2-106 Paz, G. P3/4-035 Pukazenthri, B.S. P3/4-008
Ossandón, E. P5/6-074 Pelletier, R.M. P1/2-041 Pullman, W. P5/6-126
Ostrowska, Z. P3/4-058 Pelletier, R.M. P3/4-044 Punab, M. P1/2-070
Ostrowska, Z. P5/6-067 Pelletier, R.M. P3/4-050 Punab, M. P1/2-094
Oszukowska, E. P5/6-003 Pelletier, R.M. P3/4-092 Punt, E.L. P1/2-034
Index of Abstract Authors

Purdy, P.H. P3/4-021 Rival, C. P5/6-049 Russell, L.D. P5/6-071
Puri, S. P5/6-102 Rizzo, M. PP-021 Ryan, L. P3/4-014
Purnell, E.T. P3/4-020 Robaire, B. PP-015 Rybalkin, S.D. P1/2-030
Purnell, E.T. P5/6-035 Robateau, A. P5/6-090 Saad, F. P3/4-055
Qing-Liu, J. P1/2-022 Robert, N.M. P5/6-049 Sadar, M.D. PP-028
Rabea, A.A. P3/4-081 Roberts, K.P. P1/2-015 Saez, F. P5/6-021
Rabea, A.A. P5/6-108 Roberts, K.P. P3/4-022 Saghirashvili, M. P5/6-055
Rajasekaran, M. P3/4-071 Robertson, D.M. P5/6-153 Saied, H. P1/2-090
Rajasekaran, M. P5/6-085 Rocha, C.C. P1/2-137 Sairam, M.R. P5/6-002
Rajasekaran, M. P5/6-072 Rocha, C.C. P3/4-102 Sakkas, D. P3/4-100
Rajasekaran, M. P5/6-080 Rochira, V. P5/6-145 Sakkas, D. P5/6-034
Rajasekaran, M. P5/6-123 Rodger, J.C. P5/6-115 Salama, N. P1/2-125
Rajasekaran, M. PP-023 Rodriguez, P.M. P5/6-038 Salama, N. P1/2-126
Ramachandran, A.V. PP-016 Rodriguez , H. P5/6-093 Salameh, W.A. P5/6-008
Ramirez, A. P1/2-073 Rodriguez , H. P5/6-074 Salas, R. P1/2-152
Ramos-Gonzalez, B. P1/2-144 Rodriguez-Rojas, R. P5/6-093 Saldanha, L.B. P1/2-122
Ramos-Gonzalez, B. P1/2-145 Roest, H.P. P1/2-144 Saleh, R.A. P5/6-024
Rana, B.K. PP-003 Rogoza, A. PP-032 Saleh, R.A. P5/6-027
Rana, B.K. P5/6-050 Rojas-Retis, J. P5/6-017 Salerni, H. P3/4-057
Ranganathan, P. P3/4-115 Romanato, M. P5/6-152 Salmi, A. P3/4-099
Ranganathan, P. P5/6-024 Romano, R. P1/2-016 Salva, A. P5/6-046
Ranganathan, P. P5/6-036 Romer, T.E. P1/2-046 Samanta, L. P3/4-047
Rattenbury, J. P1/2-019 Ron-Er, R. P5/6-062 Samir, S.O. P5/6-140
Rauch, M.C. P5/6-040 Ronquist, G. P3/4-111 Sanchez, E. P1/2-064
Ravnik, S.E. P5/6-003 Rootsi, S. P5/6-147 Sanchez, J. P1/2-073
Ravnik, S.E. P5/6-014 Rose, J. P1/2-094 Sanchez, J. P1/2-097
Rawe, V. P1/2-038 Rosenbaum, P. P3/4-050 Sanchez, R. PP-041
Raziel, A. P3/4-085 Rosenman, A. P3/4-110 Sangha, G.K. P1/2-021
Raziel, R. P3/4-111 Ross, L. P1/2-093 Santoianii, J. P1/2-109
Redkar, A.A. P1/2-051 Ross, L. P3/4-080 Santoianii, J. P3/4-096
Regadera, J. P1/2-144 Rossato, M. P3/4-083 Santos, D.R. P5/6-145
Regadera, J. P3/4-001 Rossato, M. P1/2-091 Santucci, R. P1/2-046
Regadera, J. P3/4-142 Rossato, M. P3/4-005 Sapojnikov, J. P1/2-088
Regadera, J. P3/4-143 Rossi, A. P5/6-131 Sardi, S.L.M. P1/2-076
Regadera, J. PP-002 Rossi-Ferragut, L.M. P3/4-005 Sakikaya, S. P1/2-108
Reggis, M. P5/6-150 Rossi-Ferragut, L.M. P3/4-102 Saunders, P. PP-048
Reif, S. P5/6-078 Roudebush, W.E. P3/4-020 Sauvalle, A. P1/2-098
Renehan, J. P3/4-079 Rouet, N. P5/6-035 Saxena, G. P3/4-140
Renfree, M. PP-034 Roux, M. P5/6-019 Saxena, G. P3/4-141
Repetto, H. P1/2-076 Roux, M. P1/2-103 Sbornik, M. P5/6-039
Restrepo, L.F. P1/2-073 Rovan, E. P5/6-078 Scaglia, H. PP-050
Roy, R. PP-003 Rovasio, R. P1/2-062 Schuttke, I. PP-001
Rey Valzacchi, G. P1/2-047 Rovereto, B. P1/2-043 Schachter, M. P3/4-111
Rha, K.H. P1/2-124 Royere, D. P1/2-044 Schatten, G. P1/2-053
Rha, K.H. P5/6-098 Rozsahegyi, J. P3/4-059 Schenk, J.L. P5/6-033
Richardson, R.T. PP-013 Rozsahegyi, J. P5/6-054 Schiettecatte, J. P3/4-101
Richhoff, J. P5/6-058 Rozsahegyi, J. P1/2-112 Schill, W-B. P3/4-023
Richhoff, J. PP-009 Rozsahegyi, J. P3/4-074 Schill, W-B. PP-041
Riera, F. P5/6-066 Rumpold, H. P3/4-075 Schlatt, S. PP-047
Righ, L. P1/2-142 Rumpold, H. P3/4-130 Schlegel, P.N. P1/2-102
Riley, S.C. P5/6-009 Ruskoaho, H. PP-027 Schleuning, W.D. PP-001
Ring, J. P5/6-039 Russell, L.D. PP-008 Schmeller, N. P1/2-062
Index of Abstract Authors

Schmidt, W. P3/4-110  Sikk, S.C. P5/6-025  Sudjarwo,  P5/6-028
Schoor, R. P1/2-059  Silversides, D.W. PP-007  Suh, B.Y.  P3/4-106
Schoor, R. P3/4-080  Simoni, M. PP-047  Suk, J.H.  P5/6-092
Schoysman, R. P1/2-007  Simons, D.M. P5/6-013  Sullivan, R.  P1/2-012
Schrader, M. P3/4-030  Sin, F.Y.T. P5/6-037  Sullivan, R.  PP-045
Schrader, S.M. P3/4-067  Sin, F.Y.T. P5/6-037  Sultan, A.M.  P3/4-114
Schteingart, H. P5/6-066  Sin, I.L. P5/6-037  Sundaram, K.  P1/2-140
Schubert, M. P3/4-055  Sin, I.L. P5/6-142  Suominen, J.  P3/4-142
Schulenburg, G. P1/2-143  Singh, J. P3/4-121  Sutovsky, P.  P1/2-053
Schwarzinger, F. P1/2-080  Singh, S.K. P1/2-139  Suzuki, K.  P1/2-018
Schweitzer, A. P5/6-097  Sinha Hikim, A.P. P5/6-018  Suzuki, K.  PP-012
Scolaro, J. P5/6-083  Sirard, M.A. P3/4-011  Swanson, R.J.  PP-042
Segal, S. P1/2-093  Sirard, M.A. P3/4-017  Swenson, K.  P3/4-103
Seidel, Jr., G.E. P5/6-033  Sivashanmugam, P. P2/2-093  Swerdloff, R.S.  P1/2-135
Selim, A. P5/6-146  Skakkebaek, N.E. P5/4-049  Szabá, F.  P3/4-074
Semczuk, M. P5/6-144  Skakkebaek, N.E. P5/6-069  Szabó, F.  P3/4-075
Seo, K.K. P5/6-125  Skrzypek, J. P5/6-062  Szarras-Czapnik, M.  P5/6-062
Seo, Y.K. P5/6-125  Slama, R. P5/6-003  Szymczynski, G.A.  P1/2-128
Serrour, G. P3/4-104  Slowikowska-Hilczer, J. P5/6-066  Takeyama, M.  P3/4-034
Serrano, A. P3/4-142  Slowikowska-Hilczer, J. P5/6-062  Talaat, S.M.  P5/6-100
Serrano, A. P3/4-143  Sobczynska-Tomaszewska, A. P1/2-095  Tan, J.K.  P1/2-129
Serrano, A. P2/2-093  Soffer, Y. P2/2-085  Tanabe, K.  P1/2-063
Setalá, N. P3/4-099  Soffer, Y. P3/4-111  Tanabe, K.  P1/2-075
Sezen, S.F. P5/6-124  Sofikitis, N. P5/6-043  Tanaka, H.  P3/4-060
Shah, O. P5/6-127  Sofikitis, N. P5/6-114  Tanaka, H.  P5/6-006
Shah, R. P5/6-132  Sofikitis, N. P5/6-099  Tanaka, H.  P5/6-007
Shah, S.S. P5/6-025  Sokol, R. P5/6-057  Tang, C.X.  P1/2-146
Shahab, M. P5/6-096  Soulle, M. P5/6-118  Tapia-Serrano, R.  P3/4-086
Sharma, B. P5/6-121  Spark, R.F. P5/6-118  Tapia-Serrano, R.  P5/6-152
Sharma, B. P5/6-121  Sperry, A.O. P3/4-122  Tardif, S.  P1/2-025
Sharma, B. P5/6-118  Spetz, A-C. P1/2-079  Tariq, S.  P5/6-096
Sharma, C.M. P5/6-121  Spindler, R. P5/6-089  Tarlatzis, I.  P3/4-056
Sharma, C.M. P5/6-102  Spira, A. P5/6-131  Tarusin, D.I.  P3/4-137
Sharma, R.K. P1/2-120  Spoladore, D. P3/4-038  T ejedor, C.  P1/2-064
Sharma, R.K. P3/4-032  Spyrou, G. P5/6-053  Telegrafi, S.  P1/2-127
Sharma, R.K. P5/6-024  Stachurska-Babol, M. P5/6-043  Tennisdorf, M.  P3/4-131
Sharma, R.K. P5/6-027  Stamatiadou, A. P5/6-043  Tennisdorf, M.  P3/4-132
Sharpe, R.M. PP-048  Stancati, S. P3/4-059  Tennisdorf, M.  P3/4-133
Sherif, E.H. P5/6-140  Stanton, J. P1/2-048  Tennisdorf, M.  P5/6-083
Shi, Q. P5/6-099  Steecwicz, D P3/4-043  Tennisdorf, M.  PP-029
Shibata, M. P5/6-091  Stein, M. P3/4-070  Tenover, J.L.  P5/6-082
Shin, J-H. P5/6-092  Strausburger, D. P1/2-085  Tezón, J.  P1/2-028
Shiraishi, T. PP-025  Strausberger, D. P3/4-111  Thauvin, L.  P1/2-142
Shirley, C.R. P5/6-004  Straub, B. P3/4-030  Theas, S.  P5/6-049
Shoachat, L. PP-024  Suarez, J. P5/6-153  Theodorsson, E.  P3/4-122
Short, R.V. PP-019  Suarez, J.D. P3/4-015  Therien, I.  P3/4-006
Shubert, M.A. P3/4-045  Suarez, S.S. P3/4-142  Thiem, C.  P5/6-136
Shultz, A. P1/2-093  Suárez-Quian, C.A. P3/4-143  Thomas, A.J.  P3/4-032
Sidhu, K.S. P5/6-038  Suárez-Quian, C.A. PP-002  Thomas, Jr., A.J.  P1/2-120
Siemiska, L. P3/4-058  Sudjarwo,  P5/6-025  Sudjarwo,  P5/6-028
**Index of Abstract Authors**

<table>
<thead>
<tr>
<th>Author</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanage, G.R.</td>
<td></td>
</tr>
<tr>
<td>Vanrell, J.A.</td>
<td></td>
</tr>
<tr>
<td>Vanrell, J.A.</td>
<td></td>
</tr>
<tr>
<td>Varenhorst, E.</td>
<td></td>
</tr>
<tr>
<td>Varhavský, S.V.</td>
<td></td>
</tr>
<tr>
<td>Vashishth, M.</td>
<td></td>
</tr>
<tr>
<td>Vazquez-Levin, M.</td>
<td></td>
</tr>
<tr>
<td>Vazquez-Levin, M.</td>
<td></td>
</tr>
<tr>
<td>Vazquez-Levin, M.</td>
<td></td>
</tr>
<tr>
<td>Veauve, C.</td>
<td></td>
</tr>
<tr>
<td>Veeramachaneni, D.N.R.</td>
<td></td>
</tr>
<tr>
<td>Vega, P.</td>
<td></td>
</tr>
<tr>
<td>Veilleux, S.</td>
<td></td>
</tr>
<tr>
<td>Velez de la Calle, J.F.</td>
<td></td>
</tr>
<tr>
<td>Venara, M.</td>
<td></td>
</tr>
<tr>
<td>Ventela, S.</td>
<td></td>
</tr>
<tr>
<td>Verjâjková, E.</td>
<td></td>
</tr>
<tr>
<td>Verdié, Y.</td>
<td></td>
</tr>
<tr>
<td>Verdié, Y.</td>
<td></td>
</tr>
<tr>
<td>Verheyen, G.</td>
<td></td>
</tr>
<tr>
<td>Vermaak, W.</td>
<td></td>
</tr>
<tr>
<td>Vermaeve, V.</td>
<td></td>
</tr>
<tr>
<td>Verville, N.</td>
<td></td>
</tr>
<tr>
<td>Vieira, M.E.M.</td>
<td></td>
</tr>
<tr>
<td>Vierula, M.</td>
<td></td>
</tr>
<tr>
<td>Viger, R.S.</td>
<td></td>
</tr>
<tr>
<td>Viktor, E.</td>
<td></td>
</tr>
<tr>
<td>Vilanova, L.T.</td>
<td></td>
</tr>
<tr>
<td>Villemur, J.</td>
<td></td>
</tr>
<tr>
<td>Vincent, M.C.</td>
<td></td>
</tr>
<tr>
<td>Vinci, M.C.</td>
<td></td>
</tr>
<tr>
<td>Vitale, M.L.</td>
<td></td>
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<tr>
<td>Vitale, M.L.</td>
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<tr>
<td>Vitale, M.L.</td>
<td></td>
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<tr>
<td>Vítetá, D.</td>
<td></td>
</tr>
<tr>
<td>Vízute, A.M.</td>
<td></td>
</tr>
<tr>
<td>Vlasblom, M.</td>
<td></td>
</tr>
<tr>
<td>von Eckardstein, S.</td>
<td></td>
</tr>
<tr>
<td>von Eckardstein, S.</td>
<td></td>
</tr>
<tr>
<td>von Eckardstein, S.</td>
<td></td>
</tr>
<tr>
<td>Vreeburg, J.T.M.</td>
<td></td>
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<tr>
<td>Vreeburg, J.T.M.</td>
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<tr>
<td>Vukovic, I.</td>
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<td>Vukovic, I.</td>
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<td>Vukovic, I.</td>
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<td>Wada, H.</td>
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<tr>
<td>Wade, M.</td>
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<tr>
<td>Wagner, M.</td>
<td></td>
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<tr>
<td>Walczak-Jedrzejewska, R.</td>
<td></td>
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<tr>
<td>Walker, I.</td>
<td></td>
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<tr>
<td>Walker, K.</td>
<td></td>
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<tr>
<td>Walker, M.</td>
<td></td>
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<tr>
<td>Wang, C.</td>
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<tr>
<td>Wang, F.N.</td>
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<td>Wang, F.N.</td>
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<tr>
<td>Wang, P.Y.</td>
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<tr>
<td>Wang, R.</td>
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<tr>
<td>Wang, S.L.</td>
<td></td>
</tr>
<tr>
<td>Warchol, J.B.</td>
<td></td>
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<tr>
<td>Waring, A.</td>
<td></td>
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<tr>
<td>Watanabe, J.</td>
<td></td>
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<tr>
<td>Watanabe, M.</td>
<td></td>
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<tr>
<td>Wattage, A.</td>
<td></td>
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<tr>
<td>Webb, C.</td>
<td></td>
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<tr>
<td>Weber, M.</td>
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<td>Wu, A.T.H.</td>
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<td>XLI, J.P.</td>
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<td>Yamada, S.</td>
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<td>Yamamoto, Y.</td>
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<td>Yamanaka, M.</td>
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</tr>
</tbody>
</table>
### Index of Abstract Authors

<table>
<thead>
<tr>
<th>Author</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang, D.Y.</td>
<td>P3/4-082</td>
</tr>
<tr>
<td>Yano, M.</td>
<td>P5/6-079</td>
</tr>
<tr>
<td>Yasuda, K.</td>
<td>P5/6-079</td>
</tr>
<tr>
<td>Yavetz, H.</td>
<td>P3/4-035</td>
</tr>
<tr>
<td>Yeung, C.H.</td>
<td>P3/4-016</td>
</tr>
<tr>
<td>Yilmaz, A.F.</td>
<td>P1/2-108</td>
</tr>
<tr>
<td>Yoge, L.</td>
<td>P3/4-035</td>
</tr>
<tr>
<td>Yomogeda, K.</td>
<td>P3/4-034</td>
</tr>
<tr>
<td>Yoon, S.R.</td>
<td>P1/2-041</td>
</tr>
<tr>
<td>Yoon, S.R.</td>
<td>P3/4-092</td>
</tr>
<tr>
<td>Yoshida, K.</td>
<td>P5/6-079</td>
</tr>
<tr>
<td>Yoshiike, M.</td>
<td>P1/2-075</td>
</tr>
<tr>
<td>Yoshiike, M.</td>
<td>P3/4-095</td>
</tr>
<tr>
<td>Yoshiike, M.</td>
<td>PP-043</td>
</tr>
<tr>
<td>Yoshitake, N.</td>
<td>P5/6-007</td>
</tr>
<tr>
<td>Yotsukura, M.</td>
<td>P1/2-077</td>
</tr>
<tr>
<td>Yotsukura, M.</td>
<td>P1/2-078</td>
</tr>
<tr>
<td>Young, L.</td>
<td>P3/4-121</td>
</tr>
<tr>
<td>Young, L.G.</td>
<td>P1/2-008</td>
</tr>
<tr>
<td>Younis, N.</td>
<td>P5/6-073</td>
</tr>
<tr>
<td>Yssel, C.F.</td>
<td>P1/2-103</td>
</tr>
<tr>
<td>Yu, Y.</td>
<td>P3/4-004</td>
</tr>
<tr>
<td>Yu, Y.</td>
<td>PP-038</td>
</tr>
<tr>
<td>Yuen, W.</td>
<td>P3/4-103</td>
</tr>
<tr>
<td>Zadyan, S.S.</td>
<td>P3/4-137</td>
</tr>
<tr>
<td>Zaky, S.M.</td>
<td>P3/4-088</td>
</tr>
<tr>
<td>Zalinkevicius, R.</td>
<td>P5/6-069</td>
</tr>
<tr>
<td>Zalkalns, Y.</td>
<td>P5/6-148</td>
</tr>
<tr>
<td>Zarmakoupis, C.N.</td>
<td>P3/4-065</td>
</tr>
<tr>
<td>Zarmakoupis-Zavos, P.N.</td>
<td>P1/2-101</td>
</tr>
<tr>
<td>Zarmakoupis-Zavos, P.N.</td>
<td>P3/4-065</td>
</tr>
<tr>
<td>Zarmakoupis-Zavos, P.N.</td>
<td>P3/4-114</td>
</tr>
<tr>
<td>Zarrilli, S.</td>
<td>P1/2-110</td>
</tr>
<tr>
<td>Zarrilli, S.</td>
<td>P5/6-101</td>
</tr>
<tr>
<td>Zavos, P.M.</td>
<td>P1/2-101</td>
</tr>
<tr>
<td>Zavos, P.M.</td>
<td>P3/4-065</td>
</tr>
<tr>
<td>Zavos, P.M.</td>
<td>P3/4-114</td>
</tr>
<tr>
<td>Zazulevsky, S.G.</td>
<td>P3/4-076</td>
</tr>
<tr>
<td>Zazulevsky, S.G.</td>
<td>P5/6-135</td>
</tr>
<tr>
<td>Zhan, P.</td>
<td>P3/4-132</td>
</tr>
<tr>
<td>Zhan, P.</td>
<td>PP-029</td>
</tr>
<tr>
<td>Zhang, G.Q.</td>
<td>P1/2-146</td>
</tr>
<tr>
<td>Zhang, H.</td>
<td>P1/2-020</td>
</tr>
<tr>
<td>Zhang, H.</td>
<td>P1/2-079</td>
</tr>
<tr>
<td>Zhang, H.M.</td>
<td>P1/2-146</td>
</tr>
<tr>
<td>Zhang, J.</td>
<td>P1/2-079</td>
</tr>
<tr>
<td>Zhang, J.W.</td>
<td>P3/4-090</td>
</tr>
<tr>
<td>Zhang, M.</td>
<td>P1/2-079</td>
</tr>
<tr>
<td>Zhang, X.R.</td>
<td>P3/4-037</td>
</tr>
<tr>
<td>Zhang, X.R.</td>
<td>P3/4-038</td>
</tr>
<tr>
<td>Zhang, Y.H.</td>
<td>PP-013</td>
</tr>
<tr>
<td>Zhang, Z.</td>
<td>P1/2-079</td>
</tr>
<tr>
<td>Zhang, Z.</td>
<td>PP-034</td>
</tr>
<tr>
<td>Zhao, J.R.</td>
<td>P3/4-090</td>
</tr>
<tr>
<td>Zhao, M.</td>
<td>P5/6-004</td>
</tr>
<tr>
<td>Zheng, Y.</td>
<td>P1/2-039</td>
</tr>
<tr>
<td>Zhu, W-J.</td>
<td>P1/2-022</td>
</tr>
</tbody>
</table>