Schedule At A Glance

Thursday, March 26, 1998
8:00 am  Andrology Laboratory Workshop (concludes at 5:00 pm)
12:00 noon Executive Council Meeting (concludes at about 10:00 pm)

Friday, March 27, 1998
8:00 am  Postgraduate Course (concludes at 5:00 pm)
5:30 pm  Opening Reception  Sponsored by SmithKline Beecham Pharmaceuticals
7:00 pm  Welcome
7:15 pm  Distinguished Andrologist Award Presentation
7:30 pm  Serono Award Lecture
• The Molecular Biology of the Zona Pellucida
  Jurrien Dean, MD

Saturday, March 28, 1998
8:00 am  AUA Lecture
• Impotence and Nitric Oxide: The Future
  Jacob Rajfer, MD
8:55 am  Distinguished Service Award Presentation
Sponsored by Genetics & IVF Institute
9:05 am  Buckeye State-of-the-Art Lecture
• Fertilization: Ignition of the First Cell Cycle
  Sally D. Perreault, PhD
10:00 am  Coffee Break in the Exhibit Hall
10:30 am  Oral Session I: Testis (Simultaneous session)
10:30 am  Oral Session II: Sperm Function (Simultaneous session)
12:00 noon Lunch
Women In Andrology Meeting & Luncheon
• Juggling Professional and Personal Obligations During the Long-Term Illness of a Loved One... and Afterwards
  Patricia Fail, PhD
1:30 pm  Symposium: Male Reproductive Aging
• Androgen Replacement in Older Men: Should We or Shouldn't We?
  J. Lisa Tenover, MD, PhD
• Male Reproductive Tract Aging
  Barry Zirkin, PhD
• Neuroendocrine Facets of Male Reproductive Aging
  Johannes Veldhuis, MD
3:30 pm  Refreshment Break in the Exhibit Hall
4:00 pm  Oral Session III: Sperm Cyropreservation
5:00 pm  Poster Session I (concludes at 7:00 pm)
Wine and Cheese Reception sponsored by TheraTech, Inc.
7:30 pm  Banquet
Sponsored by ALZA Pharmaceuticals

Sunday, March 29, 1998
8:00 am  ASA State-of-the-Art Lecture
• Using a Genetic Model of Male Infertility: What Good is a Sterile Mouse?
  Patricia Olds-Clarke, PhD
8:55 am  Young Andrologist Award Presentation
Sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.
9:00 am  Oral Session IV: Hormonal Regulation
10:30 am  Pharmacia & Upjohn Clinical Debate
• Is ICSI a Genetic Time Bomb?
  - Yes.
  - No; it is Safe and Effective
  Peter Schlegel, MD
  - Ethics and Andrology in the 21st Century
  Glenn McGee, PhD
12:00 noon Lunch
Editorial Board Luncheon
Laboratory Science Forum Meeting & Lunch
• The Role of the Andrologist/Embryologist in a Successful ART Program
  David S. Karabinus, PhD
1:30 pm  Symposium: Gene Knock-outs and Male Reproduction —
Clinical Implications
• Reproductive Consequences of an IGF-1 Null Mutation
  Anthony R. Bellvé, PhD
• Genetic Defects in Mouse and Man that Affect Gonadal Development and Function
  Sally Ann Camper, PhD
• Steroidogenic Factor 1 Plays Multiple Roles in Reproduction
  Keith L. Parker, PhD
3:30 pm  Refreshment Break in the Exhibit Hall
4:00 pm  Awards Ceremony & Business Meeting
5:00 pm  Poster Session II (concludes at 7:00 pm)
Wine and Cheese Reception sponsored by TheraTech, Inc.
8:00 pm  Student Colloquium & Soirée
Sponsored by California Cryobank, Inc.
• The XY Files: Ontogeny of an Andrologist
  Stuart E. Ravnik, PhD

Twenty-Third Annual Meeting of the American Society of Andrology
President's Welcome

Welcome to the 23rd Annual Meeting of the American Society of Andrology. We are all looking forward to having a great meeting here in southern California! The Society could not hold such meetings without the willingness and hours of hard work of the Local Arrangements, Program and Postgraduate Course Committees. We should all express our thanks to Dr. Shalender Bhasin, chair of the Local Arrangements Committee, Dr. Barry Hinton, chair of the 1998 Program Committee, and Dr. Stuart Howards, chair of the Postgraduate Course Committee, for all their efforts on our behalf. The location and programs are excellent, and we will leave Long Beach not only with pleasant memories, but with updated information and new ideas about the clinical and basic aspects of Andrology.

As our Annual Meeting depends on willing volunteers within the Society, it also depends on the contributions of our industry sponsors and exhibitors. Sponsors who have generously donated to support our meeting, whether for specific events or for general support, are noted throughout the program book, and I thank them here, collectively, as well. Also, I urge you all to visit the exhibit area during the meeting not only to learn about the development of new products, but to express to the exhibitors your appreciation for their presence. We all need each other to make our meetings successful.

The Society's officers, members of the Executive Council, committee chairs, Journal and newsletter editors, Androlog monitors and the Holland-Parlette staff, who manage our business office, all have provided excellent contributions to make this a functioning, active society. They have my sincere respect and appreciation.

Finally, I would like to thank the membership of the Society for the opportunity to serve as your president. Being president of the ASA has not only been an honor, but it has provided me the opportunity to work with a wide range of our membership and to see personally the dedication of so many. Thank you for all your efforts.

Terry T. Turner, PhD
President

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|-----------|-------------------------------|-----------------------------------|-------------|------------------------------------------|-------------------------------|-----------------------------|--------|----------------|-------------------|-------------------------|-----------------------------|-------------------------|----------------|----------------------------|-------------------------------|----------------|--------------------------|------------------|------------------------|----------------|-----------------------|-------------------|-------------------|------------------------|
March is a great month to hold the ASA meeting in Long Beach; the weather should be temperate, and with the Grand Prix and Easter around the corner, the city will have a festive spirit. The Hyatt Regency is a lovely property on the waterfront, a few blocks from the Queen Mary. The city has many attractions in its vicinity for spouses and children, including Disneyland, Universal Studios, Rodeo Drive, Knott's Berry Farm, Magic Mountain, and the Getty Museum. Therefore, the ASA meeting provides an excellent opportunity to combine great science with family fun.

Barry Hinton has put together an outstanding scientific program. The opening reception, wine and cheese receptions at the poster sessions and the banquet should facilitate socialization and networking. The Society gratefully acknowledges the support of ALZA Corporation, SmithKline Beecham Pharmaceuticals and TheraTech, Inc. for their generous support of these social events.

Our gratitude is due to members of the Local Arrangements Committee, as well as Sarah Lee and Carol Parlette in the business office, for their efforts in making it all come together. This tradition of volunteerism is what gives ASA meetings a uniquely personal flavor. Because of the location and the excellent program, we expect terrific attendance in Long Beach, so be sure to make your reservations early! It promises to be another wonderful meeting.

Shalender Bhasin, MD
Chair, 1998 Local Arrangements Committee

Local Arrangements Committee: Stefan Arver, Nestor Gonzales-Cadavid, Cora Kikunaga, Ma Kun, Wael Salameh, Amiya Sinha-Hikim, Ronald S. Swerdloff, Wayne Taylor, Christina Wang, Shalender Bhasin (chair).

American Society of Andrology

Officers
President ...................... Terry T. Turner, PhD
Vice President .............. Richard V. Clark, MD, PhD
Past President .............. Arnold M. Belker, MD
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Secretary ...................... Rex A. Hess, PhD
Executive Director .......... Carol Holland Parlette, MPH

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Alvin M. Matsumoto, MD; Craig Niederberger, MD; Deborah A. O’Brien, PhD; Peter N. Schlegel, MD;
Steven M. Schrader, PhD; Susan S. Suarez, MS, PhD; Jacquetta M. Trasler, MD PhD; Barry R. Zirkin, PhD

Committee Chairs

Andrology Labs .......... Christopher J. DeJonge, PhD, HCLD
Awards ......................... Stuart E. Ravnik, PhD
Bylaws ........................ Matthew P. Hardy, PhD
CME Liaison ................... Hugh C. Hensleigh, PhD
Finance ......................... Joel L. Marmor, MD
Future Meetings .......... Bernard Robaire, PhD
Industrial Relations ....... Kenneth P. Roberts, PhD
International Liaison ....... Barry T. Hinton, PhD
ISA 2001 ...................... Carlos R. Morales, DVM, PhD
Laboratory Science Forum .... Carol S. Sloan, MS
1998 Local Arrangements ...... Shalender Bhasin, MD
1999 Local Arrangements ...... Arnold M. Belker, MD
2000 Local Arrangements .... Robert A. Newton, MD

Long-Range Planning .... Richard V. Clark, MD, PhD
Membership ................... Susan H. Benoff, PhD
Newsletter Editors .......... Don F. Cameron, PhD
Executive Director .......... Carol Holland Parlette, MPH
Nominating .................... Alvin M. Matsumoto, MD
Placement Service Coordinator ... Pasquale Patrizio, MD
1998 Postgraduate Course ... Stuart S. Howards, MD
1999 Postgraduate Course ... Christina Wang, MD
1998 Program .................. Barry T. Hinton, MD
1999 Program .................. William J. Brenner, MD, PhD
Publications ................... Lonnie D. Russell, MS, PhD
Society Liaison ............... Harris M. Nagler, MD
Student Affairs ............... Don F. Cameron, PhD

Journal of Andrology

Editorial Office: 4-144 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455
Phone: (612) 625-1488 • Fax: (612) 625-1163 • Email: andrology@med.umn.edu
Editors-in-Chief: David W. Hamilton, PhD and Jon L. Pryor, MD • Editorial Assistant: Lauren Fox
Associate Editors: Shalender Bhasin, MD, Christopher DeJonge, PhD, Wayne J. Hellstrom, MD
International Associate Editors: David J. Handelsman, MD, PhD, Hector Chemes, MD, PhD, Gerhard F Weinbauer
General Information

Registration
All meeting attendees are encouraged to register in advance whenever possible. Registration forms are available by contacting the American Society of Andrology:
74 New Montgomery, Suite 230
San Francisco, CA 94105
Phone: (415) 764-4823
Fax: (415) 764-4915
Email: 105037.1120@compuserve.com
All registrations received after Friday, February 27, 1998 (including on-site registrations) will be assessed a $45 fee.

The meeting registration and information desk will be open at the following times for on-site registration:
Thursday, March 26, 1998 7:00 am - 6:00 pm
Friday, March 27, 1998 7:00 am - 8:30 pm
Saturday, March 28, 1998 7:00 am - 5:00 pm
Sunday, March 29, 1998 7:30 am - 5:00 pm

Travel Discounts
United Airlines is offering five to ten percent off airfares to Los Angeles for attendees of the ASA Annual Meeting. To take advantage of this offer, call (800) 521-4041 and refer to Meeting ID Number 518XK — and if you make your reservations at least 60 days in advance, you'll receive an additional five percent discount. You can also receive ten percent off Alamo and Avis car rentals when you make your car reservation at the time of your plane reservation.

Airports
Long Beach is served by three airports: Los Angeles International Airport (LAX), 18 miles north; John Wayne/Orange County Airport, 25 miles south; and Long Beach Airport, which is only 15 minutes from the hotel. Taxis from LAX are approximately $40 one way and airport shuttles are about $20 per person. From Orange County, expect to pay $45 for a taxi to the hotel. Taxis from the Long Beach airport are about $15.

Hotel Accommodations
The Hyatt Regency in Long Beach, California is the headquarters for the 1998 ASA Annual Meeting and Postgraduate Course. Discounted room rates are available for ASA meeting attendees: $104 single occupancy, $119 double occupancy, $134 triple occupancy, $149 quadruple occupancy. Regency Club accommodations are available. These rates are guaranteed only through Friday, February 27, 1998, so make your reservations as soon as possible.
Reservations may be made by calling the Hyatt reservations toll-free number: (800) 233-1234 or by contacting the hotel directly at (562) 491-1234. Be sure to mention you are attending the ASA meeting to receive the reduced rates.

Poster Sessions
Poster sessions will be 5:00 – 7:00 on Saturday and Sunday evenings in the Regency Ballroom with wine and cheese receptions supported by a grant from TheraTech, Inc.

Exhibits
An extensive exhibit hall featuring equipment and information for all andrologists will be open from 10:00 am to 7:00 pm on Saturday and Sunday in the Regency Ballroom. Refreshment breaks will be served in the exhibit hall and on Sunday there will also be a raffle among the exhibits.

Slide Preview Room
The Regency Coatroom will be available from 6:00 am until 12:00 midnight Thursday through Sunday for speakers to preview slides and prepare their presentations.

Laboratory Science Forum
The 1998 Laboratory Science Forum will be at 12:00 noon on Sunday, March 29. Luncheon tickets are available for $22. The topic this year is The Role of the Andrologist/Embryologist in a Successful ART Program.

Women In Andrology
The Women In Andrology group will hold its annual business meeting on Saturday, March 28 at 12:00 noon in the Beacon A room of the Hyatt Regency. Following the WIA meeting, Dr. Patricia Fail will address the group on Juggling Professional and Personal Obligations During the Long-Term Illness of a Loved One... and Afterwards. Tickets for the luncheon are available for $25.

Student Colloquium & Soirée
The 1998 ASA Student Colloquium, supported by an educational grant from California Cryobank, Inc., will feature Dr. Stuart Ravnik. His talk The XY Files: Ontogeny of an Andrologist will be at 8:00 pm on Sunday, March 29 in the Seaview A room of the Hyatt. The annual Student Soirée will be held immediately following the Colloquium. Both events are free of charge and everyone is welcome to attend.

ASA Home Page
Perhaps one of the most challenging tasks for the American Society of Andrology is to provide a meeting that satisfies the interests of such a diverse group of andrologists. We hope you will find something in this year's meeting that gets the creative juices flowing, that makes you want to get back to the lab and try out new ideas, or to consider new approaches to solve clinical problems. The Annual Meeting of the American Society of Andrology is a wonderful opportunity for students and veterans of Andrology to interact, to discuss new ideas, and to form collaborations. The mission of the 1998 Program Committee has been to have an excellent program. Through the committee's outstanding efforts this is reflected in the meeting.

It is a privilege to have Dr. Jurrien Dean as the Serono Lecturer. Dr. Dean has made significant contributions to our understanding of the molecular architecture and function of the zona pellucida in sperm-egg interactions. Dr. Dean will present a lecture entitled *The Molecular Biology of the Zona Pellucida*. The Society is also honored to have Dr. Jacob Rajfer as this year's American Urological Association lecturer. Dr. Rajfer has had a long-term interest in male impotence and the mechanisms that underlie this clinical problem. The title of his presentation is *Impotence and Nitric Oxide: The Future*.

The Society is fortunate to have support from individuals and corporations that allow the Program Committee to invite speakers to present state-of-the-art lectures. The Buckeye Lecture (sponsored by Buckeye Urology and Andrology, Inc.) will be presented by Dr. Sally Perreault from the US Environmental Protection Agency. Dr. Perreault has been actively investigating the process of sperm-egg interactions following the fertilization event. The title of her presentation is *Fertilization: Ignition of the First Cell Cycle*. Dr. Trish Olds-Clark from Temple University will be presenting the American Society of Andrology State-of-the-Art Lecture entitled *Using Genetic Models of Male Infertility: What Good is a Sterile Mouse?* Dr. Olds-Clark is well known for her studies on the t-haplotype and sperm motility.

One of the highlights of the Annual Meeting is the clinical debate sponsored by Pharmacia & Upjohn. This year we have a provocative debate focusing on whether ICSI is a genetic time bomb. Dr. Arnold Belker has agreed to chair the debate between Drs. Dolores Lamb, Peter Schlegel, and Glenn McGee. An important part of the debate is the opportunity for the audience to take an active part in the discussion.

Two diverse symposia, but of paramount interest to many Andrologists, have been organized. The first symposium tackles issues concerning male reproductive aging. Drs. Barry Zirkin, Lisa Tenover, and Johannes Veldhuis have agreed to present their latest studies and ideas. The second symposium examines the use of gene knock-out models to study male reproduction. This is a very exciting and growing area of male reproductive biology. Drs. Sally Ann Camper, Keith Parker, and Tony Bellvé will share with you how gene knock-outs can affect development and function of the male reproductive tract.

Students of the American Society of Andrology play a critical role in the success of the Annual Meeting and in the Society itself. Each year the Student Affairs Committee organizes a Student Colloquium and Soirée. This year Dr. Stuart Ravnik has agreed to speak on the topic *The XY Files: Ontogeny of* Continued on page five —

### Past Presidents of the American Society of Andrology

<table>
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<tr>
<th>Year</th>
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<tr>
<td>1975-1977</td>
<td>Emil Steinberger</td>
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<td>William D. Odell</td>
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<td>Larry L. Ewing</td>
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<td>C. Wayne Bardin</td>
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Dr. Jurrien Dean received his MD degree from the College of Physicians and Surgeons at Columbia University in New York, and spent two years as an intern and an assistant medical resident at The Presbyterian Hospital, New York. Dr. Dean joined the intramural research program at the National Institutes of Health in 1981 and is currently Chief of the Laboratory of Cellular and Developmental Biology, NIDDK.

Much of Dr. Dean's work has focused on defining the molecular biology of the mouse and human zona pellucida, and the function of the zona pellucida in sperm-egg interactions. Over the last decade he has characterized the genes that encode each of the three zona proteins and has investigated the molecular basis of their coordinate, oocyte-specific expression. His laboratory is currently using mouse genetics to determine the role of individual zona proteins in sperm binding and create mouse models with mutations that affect fertility.

Dr. Dean has been the recipient of the Commendation Medal and the Outstanding Service award given by the US Public Health Service, and has served on numerous Study Sections at the National Institutes of Health. He is currently the President of the NIDDK/NIAMS Assembly of Scientists. The Society is honored to have Dr. Dean as our Serono Lecturer.

### Serono Lectureship Recipients

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<td>Frank S. French</td>
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<td>Kevin J. Catt</td>
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<td>1988</td>
<td>Roger V. Short</td>
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<td>Patrick C. Walsh</td>
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The Serono Lectureship is sponsored by Serono Laboratories, Inc.

Continued from page four — an Andrologist at the Colloquium. The Soirée provides an excellent venue for students to meet with senior and well-established investigators.

An event that has received considerable praise by members of the Society and has grown in participation each year is the Women in Andrology Luncheon. This event is open to all and features a keynote speaker; Dr. Patricia Fail will speak on *Juggling Professional and Personal Obligations During the Illness of a Loved One*. Andrology laboratory staff have the opportunity to participate in the Andrology Laboratory Workshop and in the Laboratory Science Forum. The topic to be covered at the Workshop on Thursday will be *In Search of the Elusive Sperm*.

The oral and poster sessions are an important part of the Annual Meeting and provide an opportunity for members and guests of the Society to present and discuss the latest findings from their laboratories and clinical practice. Many collaborations and new ideas have originated in these sessions. This year, we are experimentally holding one simultaneous oral session. Please share your thoughts on this format with me or the ASA staff.

Finally, to add to the scientific aspects of the meeting, the Local Arrangements Committee has planned an excellent social program. We hope you can join us and look forward to seeing you there!

Barry T. Hinton, PhD  
Chair, 1998 Program Committee

Program Committee: Leland Chung, Gail Cornwall, Charles Flickinger, Hugh Hensleigh, Peter Schlegel, Barbara Sanborn, Jacquetta Trasler, Johannes Veldhuis, Barry Hinton (Chair).

Abstract Review Committee: Grace Centola, Gail Cornwall, Nina Davis, Joanna Ellington, Charles Flickinger, Duane Garner, Ron Lewis, Susan Rothmann, Barbara Sanborn, Peter Schlegel, Jacquetta Trasler, Leona Young, Barry Hinton (Chair).
Ryuzo Yanagimachi, PhD is the recipient of the Distinguished Andrologist Award for 1998. Dr. Yanagimachi began his training in reproductive biology at Hakkaido University in Japan, receiving a DSc degree in 1960. He moved to the Worcester Foundation to work with previous Distinguished Andrologist Dr. M.C. Chang for three fruitful years and then returned to Hakkaido University as Lecturer in Embryology. Two years later, Dr. Yanagimachi began his faculty appointment in the Department of Anatomy and Reproductive Biology at the University of Hawaii, where he remains today.

Dr. Yanagimachi's contributions to the field of Reproductive Biology and, indeed, to Andrology are enormous. Few individuals in modern history have had as significant an impact on the development and direction of their field as has Dr. Yanagimachi. His research is highly significant and well-regarded, receiving numerous awards including, in 1996, the International Prize for Biology. In over 300 publications, Dr. Yanagimachi's contributions are milestones in Andrology: first demonstration of mammalian sperm capacitation in vitro, first description of the manner of sperm entry into living mammalian ova, discovery of sperm hyperactivation, first demonstration of the importance of Ca++ in the sperm acrosome reaction and oocyte activation in mammal, first intracytoplasmic sperm injection in mammals, first description of the usefulness of zona-free hamster oocytes and many, many others. In addition, he has also contributed in a major way to studies on the biology of spermatozoa within the female genital tract, investigation into the stability/instability of mammalian sperm nucleus, and has demonstrated that the spermatid nucleus is potentially ready for participating in embryonic development. Dr. Yanagimachi's reviews of sperm biology are considered required reading for many Andrologists and his comparative work on multiple species (from the well known rodents to less well studied species such as bats and dolphins) should serve as a challenge to us all.

In his typical low key fashion, Dr. Yanagimachi describes his thoughts for the future. “My interest in life’s beginning goes back to my undergraduate days. Although artificial reproduction without germ cells may be possible in the future, reproduction via germ cells will remain the most effective means of continuing life of most animals (including our own species) on the surface of the earth. Compared with Mother Nature, our past and recent ‘triumphs’ in reproductive biology and technologies are trivial. We must keep learning from Mother Nature’s ways over millions of years in order to better control/manage gamete formation/functions without serious errors. It is obvious that female and male contribute equally, at least genetically, to following generations. Male gametes (spermatozoa) are made for female gametes (eggs) and vice versa. Parthenogenesis, androgenesis and cloning may technically become possible during the 21st century, but would it be desirable to live in the world without the union (love) of gametes (male and female partners)".

Dr. Yanagimachi has been a mentor to those who have worked with him directly as well as mentor to all of us through the written and oral description of his research. For unparalleled excellence in Andrology research, his many contributions to our understanding of the ability of the spermatozoon to fertilize, and his challenges to us as students of Biology, the American Society of Andrology is delighted and privileged to present Dr. Ryuzo Yanagimachi with the 1998 Distinguished Andrologist Award.

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**Distinguished Andrologists**

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The Distinguished Andrologist Award is sponsored by the American Society of Andrology.
1998 Distinguished Service Award

Rupert P. Amann, PhD is the 1998 recipient of the Distinguished Service Award. He received a BS in Dairy Husbandry from the University of Maine, and MS and PhD degrees in Dairy Science from Pennsylvania State University. After attaining the rank of Professor at Penn State, he moved to Colorado State University in 1979 and was head of the Department of Physiology from 1989 to 1995, when he became Emeritus Professor. Dr. Amann has also been Vice President for Research at BioPore, Inc. since 1989.

Dr. Amann is known internationally for his research on male reproductive physiology and endocrinology. He has made significant contributions to basic research using 13 species including humans, farm, and laboratory animals, utilizing the entire spectrum from whole animal to cellular approaches. His dedication to the field of Andrology is reflected in his many areas of service. He has been an effective mentor to numerous graduate students, a number as active investigators in Andrology. He is a founding member of both the Society for the Study of Reproduction and the American Society of Andrology and has served ASA in many important capacities: as a member of the Executive Council and the Editorial Board, as Chair of Local Arrangements, Finance and Program Committees, and as Secretary, Vice-President and President (1989–1990).

Dr. Amann has received numerous awards in recognition for his scientific contributions and many efforts on behalf of the discipline of reproductive biology. These include the American Society of Animal Science Award, the Pennock Distinguished Service Award from Colorado State University and ASA's Distinguished Andrologist Award. He has been a prolific researcher, writer, and sought-after speaker at national and international meetings.

Dr. Amann also is a keen student of the history of Andrology. “Andrology went through an infantile phase of development for over six millennia and I was fortunate to be present as the discipline went through puberty and reproductive capacity rapidly developed. As a student in the mid 1950s, it was possible to draw on emerging technologies which...opened vast areas of Andrology.” Dr. Amann lists his greatest contribution as “having a small role in helping to convince policy makers that the testis is a vital organ and not to be discarded or ignored....”

The service that Dr. Amann has given to Andrology and specifically to our Society reflects his love of the work and his desire for us to enjoy our chosen discipline. As always, he challenges us with questions and suggestions for the future. “The next ten to twenty years should be exciting, but will they be rewarding in terms of real contributions to societal needs and fun to the Andrologists? Are we asking really important questions, answers which will advance the area — quantum leaps vs. reductionism? Are we avoiding tunnel vision by remembering that molecules must interact in organisms and by drawing on seemingly unrelated areas, or knowledge for other species? Our Society should take steps to ensure that potential problems are minimized by giving appropriate lectures, requiring references to the historical or organismic underpinning of work be published in our journals, and having our institutions take steps to ensure that young faculty and students are provided the resources to contribute to society via substantial service to professional organizations such as ASA.”

For his active service, insightful challenges, and enthusiastic support of Andrology, the Society is delighted to present Dr. Rupert P. Amann with the 1998 Distinguished Service Award.

Distinguished Service Award Recipients

<table>
<thead>
<tr>
<th>Year</th>
<th>Name</th>
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<tbody>
<tr>
<td>1994</td>
<td>C. Alvin Paulsen</td>
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<tr>
<td>1995</td>
<td>Andrzej Bartke</td>
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<tr>
<td>1996</td>
<td>Philip Troen</td>
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<tr>
<td>1997</td>
<td>Marie-Claire Orgebin-Crist</td>
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The Distinguished Service Award is sponsored by the Genetics & IVF Institute

Twenty-Third Annual Meeting of the American Society of Andrology
**1998 Young Andrologist Award**

Dr. William R. Kelce is the recipient of the 1998 Young Andrologist Award. Dr. Kelce received a double AB degree in Biology and Psychology from Washington University in 1981 and his MS and PhD degrees in Physiology from the University of Missouri-Columbia in 1989. He was an NIEHS NRSA fellow at Johns Hopkins University working in collaboration with Larry Ewing and Barry Zirkin on endocrine toxicology until 1992. Dr. Kelce's interests in reproductive toxicology continued in his own research program as a principal investigator in the capacity of a Project Scientist and then Research Scientist at ManTech Environmental in Research Triangle Park. Staying on in North Carolina, Dr. Kelce moved to the US Environmental Protection Agency's Health Effects Research Laboratory in 1995 as a Research Biologist and Team Leader in the Reproductive Toxicology Division. In 1996, his contributions to the academic community were rewarded with an appointment as an Adjunct Associate Professor in the Department of Pediatrics and Laboratories for Reproductive Biology at the University of North Carolina School of Medicine at Chapel Hill.

Dr. Kelce has received a number of awards, including the First Place Young Investigator Award from the Society of Toxicology and the 1996 US EPA Gold Medal Award for Scientist of the Year. His research has been consistently recognized in terms of funding from both the US EPA and the NIEHS. Dr. Kelce describes his most significant research accomplishment as the work in his laboratory which has identified a novel mechanism by which pesticides and toxic substances can produce reproductive and developmental related health effects and provides a clear demonstration that environmental toxicants can profoundly alter reproductive development.

"As these molecular toxicology studies continue, we believe they have the potential to lead to a better understanding of the molecular mechanisms responsible for normal male sexual differentiation in addition to those responsible for inducing perturbations." On the future of the ASA, Dr. Kelce says, "I would like the ASA to continue to embrace toxicology issues related to mechanisms of adverse reproductive development and/or subsequent function."

For his excellence in research and his contributions to the field of Andrology, including his recent work on environmental toxicants published in *Nature*, the American Society of Andrology is delighted to award Dr. William R. Kelce with the 1998 Young Andrologist Award.

**New Investigator Award**

The New Investigator Award is given to the ASA student member with the best abstract and research presentation at the Annual Meeting. The award encourages student members to submit and present their best work and contribute to the scientific excellence of the Society.

The recipient of the 1998 New Investigator Award will be announced at the awards ceremony at 4:00 pm on Sunday, March 29, 1998.

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**Young Andrologist Award Recipients**

<table>
<thead>
<tr>
<th>Year</th>
<th>Name</th>
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<tbody>
<tr>
<td>1982</td>
<td>L.J.D. Zaneveld</td>
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<td>1983</td>
<td>William B. Neaves</td>
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<td>1984</td>
<td>Lonnie D. Russell</td>
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<td>1985</td>
<td>Bruce D. Schanbacher</td>
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<td>1986</td>
<td>Stephen J. Winters</td>
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<td>1987</td>
<td>Ilpo T. Huhtaniemi</td>
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<td>1988</td>
<td>Larry Johnson</td>
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<td>1989</td>
<td>Barry T. Hinton</td>
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<td>1990</td>
<td>Luis Rodriguez-Rigau</td>
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<td>1991</td>
<td>Patricia M. Saling</td>
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<td>1992</td>
<td>Gary R. Klinefelter</td>
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<td>1993</td>
<td>Robert Chapin</td>
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<td>1994</td>
<td>Wayne J.G. Hellstrom</td>
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<td>1995</td>
<td>Christopher Dejonge</td>
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<td>1996</td>
<td>Paul S. Cooke</td>
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<td>1997</td>
<td>Gail A. Cornwall</td>
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The Young Andrologist Award is sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.

**New Investigator Award Recipients**

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<tr>
<th>Year</th>
<th>Name</th>
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<tbody>
<tr>
<td>1983</td>
<td>Thomas T. Tarter</td>
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<td>1984</td>
<td>Peter S. Albertson</td>
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<td></td>
<td>Randall S. Zane</td>
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<td>1986</td>
<td>Mark A. Hadley</td>
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<td>1987</td>
<td>Peter Grosser</td>
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<td>1988</td>
<td>Stuart E. Ravnik</td>
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<td>1989</td>
<td>Tracy L. Rankin</td>
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<td>1990</td>
<td>Donna O. Bunch</td>
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<td>1991</td>
<td>Robert Viger</td>
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<td>1992</td>
<td>John Kirby</td>
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<td>1993</td>
<td>Michael A. Palladino</td>
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<td>1994</td>
<td>Linda R. Johnson</td>
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<td>1995</td>
<td>Mehdi A. Akhondi</td>
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<td>1996</td>
<td>Wei Gu</td>
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<td>1997</td>
<td>Daniel B. Rudolph</td>
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<td></td>
<td>Loren D. Walensky</td>
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The New Investigator Award is sponsored by the West Michigan Reproductive Institute, P.C.
The American Society of Andrology wishes to thank the following organizations for their generous support of the 1998 Annual Meeting & Postgraduate Course.

**Gold Club**  
A minimum $10,000 contribution to an ASA endowment fund.
Buckeye Urology and Andrology, Inc.

**Silver Club**  
A minimum $5,000 contribution to an ASA endowment fund.
West Michigan Reproductive Institute, P.C.

**Sustaining Sponsor**  
A minimum $500 contribution to ASA for five or more years.
Genetics & IVF Institute
Hamilton Thorne Research
Pharmacia & Upjohn

Serono Laboratories, Inc.
Texas Institute for Reproductive Medicine and Endocrinology, P.A.

**1998 Supporters**  
A contribution of any size to support the 1998 Annual Meeting & Postgraduate Course
ALZA Pharmaceuticals
American Urological Association
California Cryobank, Inc.
Merck & Co., Inc.
Pfizer, Pharmaceuticals Group
Serono Laboratories, Inc.
SmithKline Beecham Pharmaceuticals
TAP Pharmaceuticals, Inc.
TheraTech, Inc.

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**CME Credit Information & Course Objectives**

**Annual Meeting**
Following this program, the participant should be able to:
- Discuss the structure and function of zona pellucida in sperm-egg interactions.
- Recognize the role of nitric oxide in male impotence.
- Explain the events of the cell cycle following fertilization.
- Discuss new ideas and treatments of the aging male reproductive tract.
- Identify clinical and ethical issues in assisted reproductive technologies.
- Define new advances in the use of genetic models to study male infertility.

This activity has been planned and implemented in accordance with the Essentials and Standards of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of Minnesota and the American Society of Andrology. The University of Minnesota is accredited by the ACCME to provide continuing medical education for physicians.

The University of Minnesota designates this continuing medical education activity for up to 14 hours in category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit actually spent in the educational activity.

**Postgraduate Course**
Following the Postgraduate Course on Friday, March 27, 1998, the participant should be able to:
- Explain the developmental basic science of the male reproductive tract.
- Discuss in depth the clinical issues relating to male reproduction.

This activity has been planned and implemented in accordance with the Essentials and Standards of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of Minnesota and the American Society of Andrology. The University of Minnesota is accredited by the ACCME to provide continuing medical education for physicians.

The University of Minnesota designates this continuing medical education activity for up to 6.5 hours in category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit actually spent in the educational activity.
Target Audience
Andrologists — whether clinicians, laboratory directors, biologists, technicians, researchers or students.

Course Description & CEU Credit Information
Assisted Reproductive Technologies (ARTs) are greatly advancing our ability to facilitate the reproductive process for the subfertile couple. However, some methodologies and techniques used in the rapidly advancing ARTs can be problematic. The 1998 Andrology Laboratory Workshop, In Search of the Elusive Sperm, was specifically designed to provide key background information to facilitate the understanding, current use and potential applications of some of the more advanced ART techniques. CEU Credits will be awarded pending review and acceptance by the American Board of Bioanalysis.

Financial Support
The 1998 Andrology Laboratory Workshop is supported by an educational grant from Serono Laboratories, Inc.

Thursday, March 26, 1998
Regency A
Chairs: Erma Drobnis, PhD, HCLD and Pasquale Patrizio, MD

8:00 AM  Introduction
Christopher DeJonge, PhD, HCLD, ASA Andrology Laboratories Committee Chair

8:30 AM  Pathophysiology of Human Spermatogenesis and Epididymal Function
Erna Drobnis, PhD, HCLD, University of Missouri–Columbia

9:20 AM  From Erection to Ejaculation: What Can Go Wrong?
Jacob Rajfer, MD, UCLA Medical Center

10:10 AM  COFFEE BREAK

10:30 AM  How to Search for Elusive Sperm: MESA, TESE, PESA, STW
Pasquale Patrizio, MD, Hospital of the University of Pennsylvania

11:25 AM  Laboratory Handling of Epididymal Sperm: Processing and Freezing Techniques
Teri Ord, MLT, IVF Lab Consultant

12:15 PM  LUNCH

1:30 PM  Laboratory Handling of Testicular Sperm: Spermatids and How to Find Them
Jean Liu, PhD, GBMC Fertility Center

2:15 PM  Electroejaculation and Vibrostimulation
Stephen Seager, DVM, National Rehabilitation Hospital

3:10 PM  REFRESHMENT BREAK

3:30 PM  Post-Mortem Sperm Collection
Craig Niederberger, MD, University of Illinois at Chicago

4:10 PM  Ethical Dilemmas in Post-Mortem Sperm Collection
Glenn McGee, PhD, The University of Pennsylvania

Twenty-Third Annual Meeting of the American Society of Andrology
Postgraduate Course: Developmental and Pediatric Aspects of Male Reproduction

Course Description
The 1998 Postgraduate Course is a very unique, interesting and scientifically sophisticated program on the developmental aspects of male reproduction. The course includes basic science sessions in the morning and clinical sessions in the afternoon.

Friday, March 27, 1998
Regency A

8:00 AM  Introduction
          Stuart S. Howards, MD, Postgraduate Course Chair

8:15 AM  Prostatic Development: Role of Androgens, Cell-Cell Interactions and Growth Factors
          Gerald Cunha, PhD, University of California–San Francisco

9:00 AM  Genetic and Developmental Aspects of Androgen Resistance
          Charmian Quigley, MD, Eli Lilly and Company, Indiana University School of Medicine and the Riley Hospital for Children

9:45 AM  Coffee Break

10:15 AM Developmental Neurobiology of the Male Genitourinary Tract
          Karl-Erik Andersson, MD, PhD, Lund University, Sweden

11:00 AM Genetic Control of Sexual Development
          Dolores Lamb, PhD, Baylor College of Medicine

12:00 NOON Lunch

1:15 PM  The Adolescent Varicocele: Some New Thoughts on an Old Problem
          Jon Pryor, MD, University of Minnesota Department of Urologic Surgery

2:00 PM  The Lessons of Cryptorchidism: Implications for Reproductive Biology
          Abraham Morgentaler, MD, Beth Israel Deaconess Medical Center / Harvard Medical School

2:45 PM  Refreshment Break

3:15 PM  Testicular Torsion: Experimental and Clinical Considerations
          Harris Nagler, MD, Beth Israel Medical Center

4:00 PM  Recent Advances in Our Understanding of Sex Determination and Differentiation
          Felix A. Conte, MD, University of California–San Francisco
**Annual Meeting**

**Friday, March 17, 1998**

5:30 PM  **OPENING RECEPTION**
Beacon Ballroom
Sponsored by SmithKline Beecham Pharmaceuticals

7:00 PM  **WELCOME**
Regency A
Terry T. Turner, PhD, President
Shalender Bhasin, MD, Local Arrangements

7:15 PM  **DISTINGUISHED ANDROLOGIST AWARD PRESENTATION**
Regency A

7:30 PM  **SERONO AWARD LECTURE**
Regency A
Chair: Terry T. Turner, PhD
The Molecular Biology of the Zona Pellucida
Jurrien Dean, MD, Laboratory of Cellular and Developmental Biology, NIDDK, NIH

**Saturday, March 28, 1998**

8:00 AM  **AMERICAN UROLOGICAL ASSOCIATION LECTURE**
Regency A
Chair: Richard V. Clark, MD, PhD
Impotence and Nitric Oxide: The Future
Jacob Rajfer, MD, UCLA Medical Center

8:55 AM  **DISTINGUISHED SERVICE AWARD PRESENTATION**
Regency A
Sponsored by Genetics & IVF Institute

9:05 AM  **BUCKEYE STATE-OF-THE-ART LECTURE**
Regency A
Chair: Susan S. Suarez, PhD
Fertilization: Ignition of the First Cell Cycle
Sally D. Perreault, PhD, US Environmental Protection Agency

10:00 AM  **COFFEE BREAK**
Exhibit Hall (Regency)

10:30 AM  **ORAL SESSION I: TESTIS (SIMULTANEOUS SESSION)**
Beacon B
Chairs: Rex Hess, PhD and Nina Davis, MD

1. Dance of the Meiotic Cell Cycle: Ménage-à-Trois or Pas-de-Deux? / S.E. Ravnik
2. Testicular Sperm Distribution in Azoospermia / S.J. Silber, H. Tournaye, A. Goossens, P. Nagy, P. Devroey, A.C. Van Steirteghem
3. Thyroid Hormone Regulates Mullerian Inhibiting Substance (MIS) mRNA Expression in Cultured Neonatal Rat Sertoli Cells / P.S. Cooke, N.K. Arambepola, D. Bunick
Annual Meeting

5 The Unique Structural Diversity of the Human Testis-Specific Voltage Dependent Calcium Channel (VDCC) / L.O. Goodwin, N.B. Leeds, A. Jacob, I.R. Hurley, S. Benoff

6 Phenotype of T Cell Response in Experimental Autoimmune Orchitis / P. Turek, K. Aslam, G. Benichou

10:30 AM ORAL SESSION II: SPERM FUNCTION I (SIMULTANEOUS SESSION)
Regency A
Chairs: Joanna Ellington, DVM, PhD and Ron Lewis, MD

7 Environmental Lead (Pb2+) Exposures, The Acrosome Reaction (AR) and Human Male Infertility / S. Benoff, A. Jacob, E.S. Mandel, A. Hershlag, I.R. Hurley

8 The Stimulatory Effect of GnRH Upon Sperm-Human Zona Pellucida (hZP) Binding is Mediated by a Calcium Influx / P. Morales, B. Kerr, F. Ceric, J. Scheu, C. Otero


10 Stimulation of Protein Tyrosine Phosphorylation During Capacitation-Dependent Hyperactivated Motility of Macaque Spermatozoa / M.C. Mahony, T. Gwathmey

11 Effects of Years of Vasectomy, Time Post-Surgery, and Fertility on Reactive Oxygen Species Generated by Seminal Leukocytes and Sperm of Men After Vasectomy Reversal / C.H. Muller, R.H. Shapiro, G. Chen, R.E. Berger

12 Bull Sperm Binding to Oviductal Epithelium is Ca2+-Dependent / S.S. Suarez, I. Revah, M. Lo, S. Kölle

12:00 NOON LUNCH
WOMEN IN ANDROLOGY MEETING & LUNCHEON
Beacon B
Juggling Professional and Personal Obligations During the Long-Term Illness of a Loved One... and Afterwards
Patricia Fail, PhD, Laboratory of Reproductive Biology, Research Triangle Institute

1:30 PM SYMPOSIUM I: MALE REPRODUCTIVE AGING
Regency A
Chairs: Christina Wang, MD and Bernard Robaire, PhD
Androgen Replacement in Older Men: Should We or Shouldn't We?
J. Lisa Tenover, MD, PhD, Emory University

Male Reproductive Tract Aging
Barry Zirkin, PhD, Johns Hopkins University

Neuroendocrine Facets of Male Reproductive Aging
Johannes Veldhuis, MD, University of Virginia School of Medicine

3:30 PM REFRESHMENT BREAK
Exhibit Hall (Regency)

4:00 PM ORAL SESSION III: SPERM CRYOPRESERVATION
Regency A
Chairs: Susan Rothmann, PhD and Craig Niederberger, MD

13 Mechanisms of Cryopreservation-Induced Capacitation of Bovine Sperm / J.L. Bailey, B. Bérubé

14 Two Day IUI Treatment Cycles are More Successful Than One Day IUI Cycles When Using Frozen-Thawed Donor Sperm / M. Matilsky, J. Younis, M. Ben-Ami
15 Dialysis Addition of Trehalose/Glycerol Cryoprotectant Yields Post-Thaw Mouse Sperm with Adequate Fertilizing Ability / B.T. Storey, E.E. Noiles, K.A. Thompson
16 Motility of Cryopreserved Semen Specimens Declines Over Storage Time in Subpopulations of Cryobankers / R. Dolgina, G.S. Prins

5:00 PM  → Poster Session I: Male Reproductive Tract I, Impotence, Hormonal Regulation I, Sperm Function II, Fertility and Infertility I
Regency A
Wine and Cheese Reception sponsored by TheraTech, Inc.
For a complete list of all abstracts in Poster Session I, see page 16.

7:30 PM  → Banquet
Sponsored by ALZA Pharmaceuticals
Regency A

Sunday, March 29, 1998

8:00 AM  → ASA State-of-the-Art Lecture
Regency A
Chair: Jacquetta Trasler, MD, PhD
Using a Genetic Model of Male Infertility: What Good is a Sterile Mouse?
Patricia Olds-Clarke, PhD, Temple University School of Medicine

8:55 AM  → Young Andrologist Award Presentation
Regency A
Sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.

9:00 AM  → Oral Session IV: Hormonal Regulation II
Regency A
Chairs: Terry R. Brown, PhD and William Bremner, MD, PhD
17 Differential Expression of Testosterone Biosynthetic and Metabolizing Enzymes During Pubertal Development of Rat Leydig Cells / M.P. Hardy, R.-S. Ge
18 A Preliminary Neuroendocrine Model of Feedback in the Male Reproductive Axis / J.D. Veldhuis, D.M. Keenan
19 Stage-Specific Hormonal Protection Against Heat-Induced Germ Cell Apoptosis in Rat / Y.H. Lue, A.P. Sinha Hikim, A. Leung, S. Baravarian, C. Wang, R. Swerdloff
20 Marked Supression of Dihydrotestosterone by G1198745, a Novel 5-alpha Reductase Inhibitor / R.V. Clark, D.J. Hermann

10:00 AM  → Coffee Break
Exhibit Hall (Regency)

10:30 AM  → Pharmacia & Upjohn Clinical Debate
Regency A
Chair: Arnold M. Belker, MD
Is ICSI a Genetic Time Bomb?
Yes.
Dolores Lamb, PhD, Baylor College of Medicine
No; It is Safe and Effective
Peter Schlegel, MD, The New York Hospital – Cornell Medical Center
Annual Meeting

Ethics and Andrology in the 21st Century
Glenn McGee, PhD, The University of Pennsylvania

12:00 NOON LUNCH
EDITORIAL BOARD LUNCHEON
Seaview A
LABORATORY SCIENCE FORUM MEETING & LUNCH
Regency A
The Role of A Successful Andrologist/Embryologist in a Successful ART Program
David S. Karabinus, PhD, University of Arizona Health Sciences Center

1:30 PM SYMPOSIUM II: GENE KNOCK-OUTS AND MALE REPRODUCTION — CLINICAL IMPLICATIONS
Regency A
Chair: Gail Cornwall, PhD and Andrzej Bartke, PhD
Reproductive Consequences of an IGF-1 Null Mutation
Anthony R. Bellvé, PhD, Columbia University
Genetic Defects in Mouse and Man that Affect Gonadal Development and Function
Sally Ann Camper, PhD, University of Michigan Medical School
Steroidogenic Factor 1 Plays Multiple Roles in Reproduction
Keith L. Parker, MD, PhD, University of Texas Southwestern Medical Center

3:30 PM REFRESHMENT BREAK
Exhibit Hall (Regency)

4:00 PM AWARDS CEREMONY
Regency
New Investigator Award
Sponsored by West Michigan Reproductive Institute, P.C.
Outstanding Original Research Award
Sponsored by Hamilton Thorne Research
Research Excellence Award for Female Student/Fellow
Established by Dr. Anna Steinberger and continues to be funded by society members
Student Merit Awards
Thomas S.K. Chang Student Travel Fund Awards

4:20 PM ASA BUSINESS MEETING
Regency A

5:00 PM POSTER SESSION II: MALE REPRODUCTIVE TRACT II, SPERM FUNCTION III, FERTILITY AND INFERTILITY II
Regency A
Wine and Cheese Reception sponsored by TheraTech, Inc.
For a complete list of all abstracts in Poster Session II, see page 19.

8:00 PM STUDENT COLLOQUIUM & SOIRÉE
Seaview A
Sponsored by California Cryobank, Inc.
Chair: Don F. Cameron, PhD
The XY Files: Ontogeny of an Andrologist
Stuart E. Ravnik, PhD, Texas Tech University Health Sciences Center
Poster Session I

Saturday, 5p.m. - Please have poster in place by 12:00 noon Saturday and removed by 10:00 a.m. Sunday

Male Reproductive Tract I

21 GPI-Anchored Proteins on the Sertoli Cell Surface / H.A. Watson, R.R. Fortna, S.E. Nyquist
22 The Role of Tyrosine Phosphorylation in the Regulation of Sertoli Cell Tight Junctions / M.J. Bellace, S.E. Nyquist
25 Abstract withdrawn.
28 P53-Deficiency Suppresses Germ Cell Apoptosis And Stimulates Cellular Proliferation In Vivo / Y. Lue, A.P. Sinha Hikim, T.B. Rajavashisth, C. Wang, W.E. Salameh, R.S. Swerdlaff
29 Differential Effects of Male Germ Cell Exposure to the Hypomethylating Drug, 5-Azacytidine in Rat and Mouse Models / T.E. Doerkesen, J.M. Trasler
30 Testicular Microlithiasis and Infertility is Associated with Testicular Pathology / J. Ganem, K. Workman, S.F. Shaban

Impotence

32 Interaction Between Superoxide Dismutase (SOD) and cGMP on Nitric Oxide Mediated Penile Erection in Rats / S.C. Sikka, G. Ruiz-Deya, M. Rajasekaran, W.J. Hellstrom
33 Nitric Oxide Mediated Cytotoxicity to Human Cavernosal Smooth Muscle Cells in Culture / M. Rajasekaran, J.S. Armstrong, N.A. Baratta, W.J. Hellstrom, S.C. Sikka
34 Comparison of Erectile Responses to Galanin and Galantide in the Cat / T.J. Bivalacqua, H.C. Champion, R. Wang, W.A. Murphy, D.H. Coy, P.J. Kadowitz, W.J. Hellstrom
35 Comparison of Erectile Responses to Analogs of Andrenomedullin in the Cat / T.J. Bivalacqua, H.C. Champion, R. Wang, W.A. Murphy, D.H. Coy, P.J. Kadowitz, W.J. Hellstrom
36 (TYR1)-Nociceptin and Nociceptin Have Similar Naloxone-Insensitive Erectile Activity in the Cat / H.C. Champion, T.J. Bivalacqua, R. Wang, P.J. Kadowitz, W.J. Hellstrom
37 Penile Erection Induced by Panax Notoginseng in Dogs / J.L. Yang, Z.Y. Xue, C. Han, Y. Sun, H.F. Li, H.B. Gong, Y.Y. Zhang

Hormonal Regulation I

38 Spontaneous Expression of Inducible Nitric Oxide Synthase (iNOS) in the Hypothalamus and Frontal Cortex of Aging Rats / D. Vernet, J.J. Bonavera, R. Sperdluff, N.F. Gonzalez-Cadavid, C. Wang
40 Corticosteroid Cream Pre-Treatment Decreases Severity of Skin Irritation with Androderm® in Hypogonadal Men / E. Rappaport, A. Haig, N. Asbel, T. Rallis
42 The Pesticide Lindane Inhibits Steroidogenesis and Steroidogenic Acute Regulatory (StAR) Protein in Mouse MA-10 Leydig Cells / L. Walsh, D. Stocco
Poster Session I

43 Cyproterone Acetate (CPA) 5 mg/day Plus Testosterone Enanthate (TE) 200 mg/week Does Not Induce a More Profound Sperm Suppression Compared to CPA 5 mg/day Plus TE 100 mg/week / M.C. Meriggiola, G. Di Cintio, A. Costantino, W.J. Bremner, C. Flamigni

44 Effect of Testosterone Enanthate on Structure and Nuclear DNA Content of Rhesus Monkey Prostate / T.S. Udayakumar, A. Tyagi, S.N. Das, M. Rajalakshmi

45 Effect of Testosterone Enanthate on Lipid Profile and Liver Function Tests in Monkey / A. Tyagi, M. Rajalakshmi

Sperm Function II

46 Roles of the Disintegrin Domains of the Sperm Proteins Fertilin alpha and beta in Fertilization / J.P. Evans, R.M. Schultz, G.S. Kopf

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DANCE OF THE MEIOTIC CELL CYCLE: MENAGE À TROIS OR PAS-DE-DEUX?

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Cell cycle passage requires the activity of a cyclin and a cyclin dependent kinase (Cdk) complex. For full kinase activity, the Cdk subunit must be phosphorylated, likely by another cyclin/Cdk complex known as CAK (Cdk Activating Kinase). We have previously shown that mRNA for the components of Cdk, Cyclin H and and Cdk7, are expressed in different meiotic cell types, at different points of the meiotic cell cycle. This is not true during mitosis; Cyclin and Cdk7 are always co-expressed. Recent evidence suggests that the protein MAT1, (Menage à Trois) and MAT2, (Pas-de-deux) are involved in Cdk assembly/activation factor. To understand the significance of the uncoupled expression of Cyclin H and Cdk7 during the meiosis, we have examined MAT1 during spermatogenesis. Our hypothesis is that MAT1 regulates Cdk activity during meiosis.

Western blot analysis showed that MAT1 is highly expressed in the testis as a 36 kDa protein. The apparent levels of MAT1 increase during development, peaking approximately 17 days after birth. In the testis, MAT1 protein is present in spermatogonia but also is abundant in pachytenic spermatocytes. Unexpectedly, MAT2 expression appears limited to spermatocytes in stages IV V of meiosis, coinciding with Cyclin H expression, but not Cdk7 expression. These data suggest a unique role for MAT1 during meiosis, perhaps independent of Cdk7. Ongoing experiments on the action of Cyclin H, Cdk7, and MAT1 during meiosis will provide further insight on the activation and regulation of the cell cycle proteins that control meiosis. [Supported by funds from Texas Tech University Health Sciences Center.]

TESTICULAR SPERM DISTRIBUTION IN AZOSPERMIA

S.J. Silber, Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, MO, USA and H. Toumey*, A. Goosens*, P. Nagy*, P. Devroey*, and A.C. Van Steirteghem, Centre for Reproductive Medicine, University Hospital, Dutch-Speaking Free University, Brussels, Belgium.

Objective: Men with non-obstructive azosperma caused by germinal failure can now be treated successfully in some cases using testicular sperm extraction (TESE) and ICSI. We wished to determine whether a prior diagnostic testicle biopsy analyzed quantitatively can predict success or failure of TESE-ICSI. We also wished to determine what is the threshold of quantitative sperm production in the deficient testis, below which no sperm will reach the ejaculate (azoosperma).

Method: Forty-five patients with non-obstructive azosperma caused by testicular failure underwent diagnostic testicle biopsy prior to a subsequent TESE-ICSI procedure.

Results: Men with non-obstructive azosperma caused by germinal failure had a mean of zero to 6 mature spermatids per seminiferous tubule seen on a diagnostic testicle biopsy. This compared to 17 to 35 mature spermatids per tubule in men with normal spermatogenesis and obstructive azosperma. The results of subsequent TESE-ICSI procedures are summarized below:

<table>
<thead>
<tr>
<th>Number of Sperm Found At</th>
<th>Patients</th>
<th>TESE-ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>W/Spem</td>
<td>26</td>
<td>22 (88%)</td>
</tr>
<tr>
<td>W/OSpem</td>
<td>19</td>
<td>14 (73%)</td>
</tr>
</tbody>
</table>

Incomplete testicular failure appears to involve a sparse multifocal distribution of spermatogenesis throughout the entire testicle, rather than a patchy, or local distribution in just a few areas.

THYROID HORMONE REGULATES MILLERIAN INHIBITING SUBSTANCE (MIS) mRNA EXPRESSION IN CULTURED NEONATAL RAT SERTOLI CELLS

P.S. Cooke, N.K. Arambepola*, and D. Bunic, Dept. of Vet. Biosciences, Univ. of Illinois, Urbana, IL 61802

Thyroid hormone is a major regulator of Sertoli cell development. Triiodothyronine (T3) treatment inhibits neonatal Sertoli cell proliferation in vitro. Conversely, transient neonatal hypothyroidism leads to increased Sertoli cell proliferation in vivo and increased adult testis size and sperm production. T3 treatment also stimulates production of several secretory proteins which are normally produced in increasing quantities as Sertoli cells mature. MIS, a Sertoli cell secretory protein that induces regression of the Mullerian ducts and may be inhibitory for the initiation of germ cell meiosis, shows an opposite pattern. Production of MIS mRNA, which normally decreases as Sertoli cells mature, is extended by hypothyroidism. Thus, T3 could regulate the postnatal fall in MIS mRNA, but data on hormonal regulation of MIS is fragmentary due to the lack of a rodent Sertoli cell culture where adequate expression of MIS or its mRNA can be obtained. The aim of this study was to develop a Sertoli cell culture system for examining regulation of MIS mRNA, then test the effects of T3 on MIS mRNA production. Initial studies using a serum-free cell culture system indicated that MIS mRNA production by 5-day-old rat Sertoli cells was maintained in culture. These Sertoli cells from 2-4 day-old rats were cultured for 2 or 4 days. MIS mRNA levels in these cells were low after 2 days of culture, but increased by day 4 of culture. MIS mRNA production by 2-day-old Sertoli cells was several fold greater than that seen in 5-day-old Sertoli cells. T3 (100 nM) treatment resulted in an 80% decrease in MIS mRNA levels compared to control cultures; T3-treated cultures expressed increased mullerian inhibitory mRNA compared to control cultures, showing that the T3 effect on MIS mRNA was not a non-specific one on mRNA production. This cell culture system provides the first in vitro system for examining hormonal effects on MIS mRNA production and should be useful for understanding MIS regulation in developing Sertoli cells.

1. How does T3 affect the expression of MIS mRNA?
2. What are the effects of T3 on ABP expression?
3. Are there morphologic changes in the Sertoli cells following T3 treatment?

IS INCONSTANT ASCENDING TESTIS A RISK FACTOR FOR SPERMATOGENESIS?


As the normal testis location is a scrotal position, the potential effects of testis position on sperm parameters were retrospectively evaluated.

Material and Methods: In 85 fertile and in 914 infertile men without any history of testis maldescent, testis position was recorded as low when in the bottom and as high when in the upper part of the scrotum. The patient was asked whether each testis was spontaneously and regularly ascending up to a supra-scrotal location, with the physician indicating this area with his finger. An "inconstant ascending testis" was recorded when a usual scrotal testis was spontaneously and regularly ascending up to a supra-scrotal location, who guidelines were used for semen analyses.

Results: The frequency of at least one testis in a high scrotal location did not differ between fertile (16.5%) and infertile men (17.6%). However, such a location could not be a physiological variant, as an inconstant ascending testis was more frequent in infertile men with a high than low scrotal position (right testis: OR = 2.7; 95% CI=1.7-4.2; left testis: OR = 2.9; 95% CI=1.8-4.7). The frequency of an inconstant ascending testis did not differ significantly between fertile (11.8%) and infertile men (18.3%). However, infertile men with an inconstant ascending testis had lower total sperm count (87.7±16.8 vs 118.8±35.6 x10^9, p<0.006) and motility (25.5±6.6% vs 30.4±11.5%, p<0.009) than fertile men with testes in permanent local position. These results were independent of the presence or absence of a clinical varicocele.

Discussion: A spontaneous and regular testis ascent from a usual scrotal location to a supra-scrotal position was observed in 18.3% of infertile men without any history of testis maldescent. As such an event was associated with a more impaired spermatogenesis than when testes were in permanent scrotal position, inconstant testis ascent seems to be a spermatogenesis risk factor. Impaired spermatogenesis could result from inconstant testis ascent being a crude pattern of maldescent and/or from regular testis exposure to high temperature of the supra-scrotal area.
The unique structural diversity of the human testis is of critical importance for the development of male fertility. However, the regulation of this diversity remains poorly understood. A recent study has employed a novel approach to address this question, combining immunohistochemistry with high-throughput sequencing to analyze the expression of distinct testis-specific proteins in different regions of the testis. The results indicate that the testis is a highly dynamic organ, with a complex interplay of cell types and molecular signals that determines its structural and functional diversity.

In conclusion, the study highlights the importance of considering the unique structural diversity of the human testis in the context of male fertility. Further research is needed to elucidate the mechanisms that govern this diversity and to understand its implications for human reproduction.
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EVALUATION OF 64I CONSECUTIVE CYCLES OF ARTIFICIAL INSEMINATION USING CRYOPRESERVED DONOR SEMEN (AID) M. M. Morshedi, C. Coddington, S. Voelz and S. Oehninger, The Jones Institute for Reproductive Medicine, Dept. of OB/GYN, Eastern Virginia Medical School, Norfolk, VA

OBJECTIVES: We analyzed factors affecting the pregnancy outcome in our AID program. The specific objectives were: 1) to compare the efficacy of intravaginal insemination (ICI) with intravaginal insemination (IUI); 2) to evaluate the results of one versus two inseminations per cycle; 3) to determine the cumulative probability of pregnancies for ICI and IUI cycles; 4) to investigate the relationship between female factors on the overall outcome; 3) to establish the minimal criteria for semen parameters predictive of pregnancies.

MATERIALS AND METHODS: 171 couples undergoing 641 insemination cycles were studied. A total of 236 ICI and 315 IUI cycles were performed. One insemination per cycle was carried out in 198 cycles of ICI (IC1) and in 191 cycles of IUI (IIU). Two inseminations per cycle (ICI and IUI) were performed in the remaining cases. The clinical pregnancy rate per cycle (PRU) and the cumulative pregnancy rates were determined.

RESULTS: The overall PRU was 11.2% with a miscarriage rate of 15.3%. ICI gave a PRU of 9% compared to 13% for IUI (p=0.05). The PRU for ICI, ICI, and IUI, were 9%, 13% and 14%, respectively (p=0.09). PRU for IUI vs IUI and ICI vs ICI were not different. However, the rate for IUI was significantly higher than that of ICI (13% vs 7%). Cumulative pregnancy rate for ICI was significantly higher than for ICI (p=0.01). The median (50%) cumulative rate occurred at about 4.5 months for ICI patients, but IUI patients never achieved a rate of 50%. The rate was not significantly different between IUI and IUI. However, the median rate occurred at about 5 months for IUI, compared to 6.5 months for IUI. Overall, the cumulative pregnancy rates for ICI and IUIs (one and two inseminations combined) were not significantly different. Nevertheless, the median cumulative rate (50%) for IUIs occurred at 5.8 months whereas that of ICI occurred at 7.4 months. Patients with female factor had a significantly lower pregnancy rate (11%) than those without (17%). IUI patients with female factor had a significantly lower pregnancy rate (7%) than those without (19%). Although patients who miscarried were around 3 years older than those who did not (p=0.003), age was not a factor influencing pregnancies. The minimum number of motile sperm per insemination resulting in pregnancies for ICI and IUIs were 33 and 42.4 million, respectively.

CONCLUSIONS: Compared to ICI, IUIs gave better cumulative conception rates. Female age was influential in miscarriage but not in pregnancies. Identification of a minimal threshold for the number of motile sperm may help in the selection of samples with a higher potential to achieve pregnancies.

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STIMULATION OF PROTEIN TYROSINE PHOSPHORYLATION DURING CAPACITATION-DEPENDENT HYPERACTIVATED MOTILITY OF MACAQUE SPERMATOZOA. M.C. Mahony, E. Coddington, S. Oehninger, The Jones Institute for Reproductive Medicine, Norfolk, VA

Mammalian spermatozoa exhibit characteristic motility patterns associated with the completion of capacitation. In cynomolgus monkey (Macaca fascicularis) sperm, this capacitation-related motility, termed hyperactivated motility (HA), is dependent in vitro upon the addition of exogenous cyclic nucleotide mediators, caffeine and dbcAMP. In this study we investigated the involvement of protein tyrosine phosphorylation in cAMP-stimulated HA of macaque sperm.

Methods: Semen specimens were collected in TALP-Hepes medium from proven breeder monkeys via electro-ejaculation. After washing, sperm were incubated for 2 hrs at RT. Sperm were transferred to Talp medium and incubated with and without the sperm activators, caffeine (1 mM) and dbcAMP (1 mM) for 0.5 hr at 37°C and 5% CO2 in water-saturated air. Sperm motion characteristics were assessed by computer assisted motion analysis (HTM-IVOS) and % motile sperm.

Results: Semen specimens were collected in Talp-Hepes medium from proven breeder monkeys via electro-ejaculation. After washing, sperm were incubated for 2 hrs at RT. Sperm were transferred to Talp medium and incubated with and without the sperm activators, caffeine (1 mM) and dbcAMP (1 mM) for 0.5 hr at 37°C and 5% CO2 in water-saturated air. Sperm motion characteristics were assessed by computer assisted motion analysis (HTM-IVOS) and % motile sperm.

Conclusion: These results suggest that protein tyrosine phosphorylation may represent an important signalling pathway modulating cAMP-stimulated hyperactivated motility in macaque sperm.

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EFFECTS OF YEARS OF VASECTOMY, TIME POST-SURGERY, AND FERTILITY ON REACTIVE OXYGEN SPECIES GENERATED BY SEMINAL LEUKOCYTES AND SPERM OF MEN AFTER VASECTOMY REVERSAL. C. H. Muller, R. H. Shapiro*, G. Chen* and R. E. Berger, Dept. of Urology, University of Washington, Seattle WA 98195

Production of reactive oxygen species (ROS) by seminal fluid leukocytes (WBC) can damage sperm and lead to failed fertility. We previously found that men post-vasectomy have significantly higher seminal cell ROS levels than do fertile controls. Concentrations of seminal leukocytes in the two groups were not different; and when men with leukocytospermia were excluded, the difference in ROS production remained. Either the activation status of the few leukocytes, or ROS production by damaged or immature sperm may account for this finding. We suggest that tissue damage and repair, and possible immunologic reaction to escaped spermatozoa may be reasons for increased WBC activation. If so, we expect ROS to decrease with time post-vaso, most dramatically in post-vaso men who regain fertility. To test this, we examined ROS production in washed seminal cells, and in density gradient fractions from men at various times after vasectomy reversal.

Methods: Semen samples from 44 post-vaso men (11 of them fertile) were washed 3x in BWW-BSA or prepared on 40/60% Percoll columns. All three preparations (washed seminal cells, W; 40-80% Percoll interface, I; and relatively purified sperm from 80% Percoll, P) were adjusted to 2x10^6 sperm/ml. Luminol-dependent ROS measured in relative light units (RLU) were determined in 0.1 mL aliquots at peak response (20-30 min). Final values were calculated as RLU per (10^6 WBC (W and I) or 10^6 sperm (P)). RESULTS: WBC/I tended to increase with time post-vaso, but not with yrs of vasectomy (NS). Only ROS in W was correlated with years of vasectomy (r=0.4, p<0.02). ROS in W and P steadily decreased with months post-vaso (NS). ROS in W at 1-2 months lower in fertile than other post-vaso men (p<0.02). At ten months or more, fertile post-vaso men's sperm (P) produced almost 40% less ROS than other post-vaso men's sperm (p<0.05).

Conclusion: ROS in seminal cells post-vaso is weakly increased by a longer time of vasectomy, and generally decreases within months of vasectomy reversal. Fertility post-vaso is associated with dramatically lower ROS in the I and P fractions. Determination of ROS may guide therapies and provide predictive value for pregnancy post-vasectomy reversal.

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BULL SPERM BINDING TO OVUDCTAL EPITHELIUM IS Ca^{2+}-DEPENDENT. S.S. Suarez, I. Revah*, M. Lo* and S. Kull*, Department of Anatomy, College of Veterinary Medicine, Cornell University, Ithaca, NY.

In several mammalian species, a reservoir of sperm is known to form in the uterine horn and/or isthmus of the oviduct. Sperm binding to oviducal epithelium is Ca^2+ dependent. To test this, we examined ROS production in washed seminal cells, and in density gradient fractions from men at various times after vasectomy reversal.

Methods: Semen samples from 44 post-vaso men (11 of them fertile) were washed 3x in BWW-BSA or prepared on 40/60% Percoll columns. All three preparations (washed seminal cells, W; 40-80% Percoll interface, I; and relatively purified sperm from 80% Percoll, P) were adjusted to 2x10^6 sperm/ml. Luminol-dependent ROS measured in relative light units (RLU) were determined in 0.1 mL aliquots at peak response (20-30 min). Final values were calculated as RLU per (10^6 WBC (W and I) or 10^6 sperm (P)). RESULTS: WBC/I tended to increase with time post-vaso, but not with yrs of vasectomy (NS). Only ROS in W was correlated with years of vasectomy (r=0.4, p<0.02). ROS in W and P steadily decreased with months post-vaso (NS). ROS in W at 1-2 months lower in fertile than other post-vaso men (p<0.02). At ten months or more, fertile post-vaso men's sperm (P) produced almost 40% less ROS than other post-vaso men's sperm (p<0.05).

Conclusion: ROS in seminal cells post-vaso is weakly increased by a longer time of vasectomy, and generally decreases within months of vasectomy reversal. Fertility post-vaso is associated with dramatically lower ROS in the I and P fractions. Determination of ROS may guide therapies and provide predictive value for pregnancy post-vasectomy reversal.

The fertility of bovine sperm following cryopreservation is dramatically reduced and it is not fully explained by poor post-thaw motility and morphology. We have demonstrated that immediately after thawing, a proportion of sperm are already capacitated (Cornier et al., 1997). We suggest that the sub-fertility of cryopreserved sperm is partly due to premature capacitation. To test the hypothesis that the mechanisms of capacitation induced by cryopreservation or by heparin (physiological capacitation) are different, this study compared the intracellular Ca2+ dynamics and protein profiles of fresh and cryopreserved sperm. To monitor [Ca2+]i, the fluorescence of fura-2-loaded sperm was measured at 0, 2.5, and 5h of capacitation ± 10 µg/ml heparin, prior to and after the addition Br-A23187 (n > 20 sperm from 4 ejaculates). To assess sperm protein profiles, proteins were extracted from cryopreserved cells during capacitation (+ heparin), subjected to silver nitrate staining-SOS-PAGE and western blotting using an antiphosphotyrosine antibody (n = 3). Internal [Ca2+]i seemed not to differ in fresh or cryopreserved sperm, either ± heparin before ionophore treatment (P>0.05). At 2.5 and 5h of capacitation, cryopreserved sperm ± heparin underwent a greater ionophore-induced increase in [Ca2+]i than sperm - heparin (P<0.05). This difference was only observed after 5h of capacitation in fresh sperm. At 0h, a series of tyrosine phosphorylated sperm proteins were observed ± heparin. At 2.5 and 5h, tyrosine-phosphorylated proteins were observed only from sperm ± heparin. These data demonstrate that immediately after thawing there is a population of precapacitated sperm and another more cryo-resistant population that undergoes capacitation in a manner similar to that of fresh cells (i.e. heparin and time-dependent). (Supported by the CIRB and NSERC. Thanks to the Centre d’Innovation Artificielle du Québec for semen and technical assistance.)


Cryopreserved sperm offer a low cost and space-saving means for maintenance of mutant, transgenic and knockout strains of mice. Mouse sperm are prone to membrane damage from osmotic stress during cryopreservation from osmotic imbalance on addition of pre-freeze and removal post-thaw of cryoprotectant and from imbalance due to dessication/rehydration during freezing/thawing. We showed (J. Androl. 18 [Suppl.]: P-45, 1997) that glycerol (G) plus trehalose (T) provided a useful cryoprotectant for mouse sperm in terms of plasma membrane integrity. Stress from osmotic imbalance on addition and removal of cryoprotectant is reduced by serial addition of pre-freeze (SA) and serial dilution post-thaw (SD) (Gilmore, Liu, Gao and Cistner, Hum. Reprod. 12: 112-118, 1997); in this study, we compared fertilizing ability (FA) of CD1 mouse sperm recovered post-thaw after cryopreservation with cryoprotectant 6% G/7.5% T using SA/SD, addition/removal by dialysis (DL), and DL/direct addition. FA was assayed by insemination of 10 CD1 mouse eggs in 0.5 mL drops with 2.5 x 10^5 sperm and scoring symmetrical two-cell embryos. Under these conditions, untreated fresh control sperm gave 73±5% eggs fertilized; DL/SD gave 66±7%; SA/SD gave 23±11%; DL/direct addition gave 42±5% (means±SD, n = 8). Addition of cryoprotectant by DL prefreeze and direct addition of sperm without removal of cryoprotectant post-thaw gave significantly superior FA (P<0.01) compared to DL/SD alone. Normalized to controls, DL/direct addition gave 62%. DL/direct addition using the chemically defined G/T cryoprotectant offers post-thaw FA adequate for eventual transgene transmission. (Supported by NICHD through grant HD-31757)

TWO DAY IUI TREATMENT CYCLES ARE MORE SUCCESSFUL THAN ONE DAY IUI CYCLES WHEN USING FROZEN-THAVED DONOR SPERM. M. Matilsky, J. Youde and M. Ben-Ami, Reproductive Medicine Unit, Portia Government Hospital, Tiberias, Israel.

Relatively few publications have addressed the best ways of performing donor insemination. The difference in pregnancy rates following IUI for one vs. two days in the periovulatory period has been reported as either inconsequential or favoring the use of two consecutive inseminations, 24 hours apart. Some studies, related to donor insemination, presented either small numbers of patients or less than 100 cycles per treatment group, making conclusions difficult to interpret. Two large studies (Brook et al., 1994, Deary et al., 1997), presented unbalanced study groups and one (Deary et al.), used IC3 of unwashed frozen-thawed sperm and timed the inseminations with home use urinary LH kits. Our study compared the monthly fecundity and cumulative probability of pregnancy in a large group of women (N=123) undergoing controlled ovarian hyperstimulation and one or two day inseminations with donor sperm prepared from frozen-thawed samples. The choice of single or double insemination was decided by the day of the week each patient received lCG for ovulation induction. Approximately 80% of all the patients underwent both single and double insemination treatments during the 2.5 year study period. 93 patients received single inseminations in 180 cycles, while 103 patients received double inseminations in 222 cycles. Nine clinical pregnancies were achieved in the one day group (9% cycle, 9.7% patient), while 19 pregnancies occurred in the two day group (17% cycle, 29.8% patient).Two and five spontaneous abortions occurred in the one and two day groups, yielding take home baby rates of 3.9% cycle and 15.3% cycle, respectively. The cumulative probability of conception over 15 cycles of treatment was consistently twice as high or higher for the two day group. In addition, the data revealed that a significant number of pregnancies occurred between the 7th and 12th treatment cycle/3-9 day one. As a result of this study we recommend two day IUI treatment cycles when using frozen-thawed donor sperm. A large, multicentered, prospective study would be useful in substantiating these results. Brook et al. (1994) Fertil Steril 61:308-313. Deary et al. (1997) Hum Reprod 12:1494-1496.

MOTILITY OF CRYOPRESERVED SEMEN SPECIMENS DECLINES OVER STORAGE TIME IN SUBPOPULATIONS OF CRYOBANKERS. D. Dolginas* and GS Prins. Dept of Urology, University of Illinois, Chicago, IL.

Long-term freezing of human sperm is frequently desired by men facing the possibility of sterilization, reduction of fertility potential or genetic damage. The success of the freezing-storage-thawing procedure is dependent on many factors one of those being the initial semen quality. In many cryobanks, post-thaw motility estimates are made on a frozen aliquot soon after cryopreservation to provide diagnostic information for future use. This study retrospectively determined factors affecting the prognosis of post-thaw sperm motility after various periods of cryostorage. We analyzed 31 specimens designated to be destroyed either because of patient request or due to death. Specimens were cryopreserved for 1-9 years (3.4±2.21 yrs; mean±SEM ). The clinical records, including reason for sperm banking, initial semen parameters, 24 hr post-thaw analysis and post-thaw parameters after variable periods of cryostorage were studied. In general, we observed that post-thaw motility estimates made at 24 hr after cryopreservation (P2) reliably predicted post-thaw motility years later after long-term cryostorage (P8): P8/P2 = 1.22±0.09, n=51. However, subpopulations existed which significantly affected the predictive capacity of the 24 hr post-thaw evaluation. First, initial sperm concentration was found to affect the post-thaw motility estimates over time. For patients with an initial sperm concentration between 20-60 x 10^6/ml, the P8/P2 = 0.93±0.06 (n=17) indicating that motility remained constant over time in cryostorage. In contrast, patients with oligozoospermia (<20 x 10^6/ml) had a P8/P2 = 1.77±0.43 (n=9) while patients with high sperm concentrations (>60 x 10^6/ml) had a P8/P2 = 2.60±1.39 (n=25), indicating that in both situations there is a marked motility loss over time as storage perhaps due to uneven distribution of sperm cells in cryobuffer. Secondly, patient diagnosis prior to cryostorage affected the post-thaw motility over time. Men with testicular cancer who froze specimens prior to chemotherapy possessed a P8/P2 = 1.47±0.27 (n=16) while all other patients (including patients with pre-vasectomy or other surgeries) had a P8/P2 ratio of 1.09±0.05 (n=35). In conclusion, the post-thaw motility 24 hr after cryopreservation is a useful prognostic test; however, the reliability of this parameter will depend on the initial sperm concentration as well as the presence of testicular cancer in the banking population.
The amount of testosterone (T) secreted by the Leydig cell is determined by a balance between T biosynthetic and metabolizing enzyme activities. It has been established that 3α-androstanediol (3α-DOL) is the predominant immature androgen in rats during days 20 to 40 postpartum, whereas T is the major androgen by day 56. However, the underlying changes in T biosynthetic and metabolizing enzymes during Leydig cell development, and their magnitudes, have remained unclear. The aim of the present study was to define the developmental trends for T biosynthetic and metabolizing enzymes in Leydig cells at three distinct stages of pubertal differentiation: mesenymal-like progenitors on day 21, immature Leydig cells on day 35 and adult Leydig cells on day 60. Four major androgens, androstenedione (DIONE), androstosterone (AO), T and 3α-DOL were measured in progenitor, immature, and adult Leydig cells in spent medium after 3 h in vitro. Steady-state mRNA levels and enzyme activities of biosynthetic and metabolizing enzymes were determined. LH-stimulated levels of total androgens (DIONE + AO + T + 3α-DOL) were significantly lower in progenitor, compared to immature and adult Leydig cells (P < 0.05) with 84.33 ± 8.74 ng/mL cAMP in progenitor (mean ± SE) versus 330.13 ± 44.39 in immature, and 523.23 ± 67.29 in adult. Progenitor, immature and adult Leydig cells produced different androgens, with AO being released by progenitor cells (72.06 ± 9.02% of total androgens), 3α-DOL by immature (73.33 ± 4.92%) and 74.38 ± 14.73% by adult Leydig cells (74.08 ± 14.73%). Further examination showed that this difference resulted from differential expression of T biosynthetic and metabolizing enzymes. Low levels of type III 17β-hydroxysteroid dehydrogenase (17β-HSD) mRNA and 17α-hydroxysteroid reductase activity were present in progenitor cells compared to immature and adult Leydig cells. In contrast, type I 17α-reductase (3α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD) mRNAs and enzyme activities dramatically decreased in adult Leydig cells, compared to progenitor and immature cells. Most of the T biosynthetic enzymes attained equivalent levels in immature and adult Leydig cells, but T was rapidly metabolized to 3α-DOL by high 3α-R and 3α-HSD reductive activities in the former and not the latter. The dramatic decline in 3α-R and 3α-HSD activities resulted in maximal T production in adult Leydig cells. The results indicate that the gonadotropic enzyme gene expression is not induced simultaneously, and that sequential changes in T biosynthetic and metabolizing enzyme activities result in different androgen end products being secreted by Leydig cells during pubertal development. Supported in part by the NIH grant HD-35288 (M.P.H.) and the Population Council (R.-S.G.).

A PRELIMINARY NEUROENDOCRINE MODEL OF FEEDBACK IN THE MALE REPRODUCTIVE AXIS. JD Veldhuis and DJ Hermann*, Glaxo Wellcome, Research Triangle Park, NC

Testosterone (T) is converted to the more potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase (SAR). While DHT is critical for masculinization of the genitalia in the fetus, it seems to have limited importance in adult men except for its effects in prostatic hyperplasia. We compared the hormonal effects of G198745 (GG745) to finasteride in BPH patients. GG745 is a potent inhibitor of type 1 and 2 SAR, whereas finasteride only inhibits type 2 SAR. Fifty-three BPH patients (International Prostate Symptom Score >8 and enlarged prostate on DRE) received daily oral doses of GG745, placebo, or finasteride (5mg) for 28 days in this randomized, blinded, parallel group trial. GG745 doses of 0.1, 0.5, 2.5, 5mg, and 2.5 with a 40mg loading dose were studied. DHT and T measures were taken before and after 28 days of study drug administration. GG745 groups were compared to placebo and finasteride using a general linear model with pairwise comparisons.

The results after 28 days of treatment are as follows (mean±SD):

<table>
<thead>
<tr>
<th>Group</th>
<th>T (%Reduction)</th>
<th>DHT (%Reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6</td>
<td>3221</td>
</tr>
<tr>
<td>Finasteride 5mg</td>
<td>6</td>
<td>7667*</td>
</tr>
<tr>
<td>GG745 0.1mg</td>
<td>8</td>
<td>7227*</td>
</tr>
<tr>
<td>GG745 0.5mg</td>
<td>8</td>
<td>9692#</td>
</tr>
<tr>
<td>GG745 2.5mg</td>
<td>8</td>
<td>9553#</td>
</tr>
<tr>
<td>GG745 5mg</td>
<td>8</td>
<td>9621#</td>
</tr>
<tr>
<td>GG745 10mg</td>
<td>8</td>
<td>9717#</td>
</tr>
</tbody>
</table>

*p<0.05 vs placebo, #p<0.05 vs finasteride

We concluded that dual inhibition of SAR with GG745 produced significantly greater reductions in serum DHT compared to finasteride while T remained within the normal range. GG745 is clearly capable of providing >95% reduction in serum DHT with little variability. Larger, longer duration studies are needed to determine the clinical benefit of a potent dual SAR inhibitor over a type 2 inhibitor such as finasteride.
Glycosylphosphatidylinositol (GPI) -anchored proteins constitute a class of membrane proteins that has recently gained attention in the scientific community. These proteins are sorted to the apical-lateral domain of epithelial cells, perhaps via specialized lipid rafts formed in the trans Golgi network. At the apical cell surface they reside in association with cholesterol and glycosphingolipid rich domains related to caveolae. The study reported here is an investigation of the GPI-anchored proteins found on the Sertoli cell membrane. Four-day-old Sertoli cell cultures were established from 20-day rat pups on both the standard plastic culture flask and on the porous bicameral insert. After four days of culture the cell surface proteins were biotinylated using a cell impermeant biotinylating reagent, sulfo-NHS-succinimidyl-6-(biotinamido) hexanoyl (Fischer Chem. Co.). Cells were mechanically scraped off of the plate, washed extensively and subjected to digestion by phosphatidylinositol specific phospholipase C (Boehringer Mannheim) to release GPI-anchored proteins. Following centrifugation the supernatant was collected and precipitated via acetone/methanol. The resultant protein pellet was collected and prepared for SDS-PAGE. The resultant gels were western blotted and visualized using ECL with an avidin-horse radish peroxidase kit biotinylated using here rat pups on both the network. At the apical cell surface they reside in association with cholesterol release identifiable several less prominent protein GPI-anchored protein is the 7H6 (175 kD) In epithelial cells, tight junctions (TJ) be traversed by the early separating the SERTOLI stage 4 tubule (Sertoli cell culture) created a regulated barrier to the passage of solutes, water and migrating cells. Research on mammalian cells has identified multiple TJ-associated proteins, of which ZO-1 (214 kD), ZO-2 (160 kD), and TH6 (175 kD) are reported to be tyrosine phosphorylated. Despite progress with other tissues, little is known about the molecular components of the Sertoli cell TJ or their phosphorylation status. The Sertoli-Sertoli TJ constitutes the barrier separating the basal and the adluminal compartments; it is this barrier that must be traversed by the early spermatocyte. The purpose of this study was to examine tyrosine phosphorylation patterns on putative tight junction proteins. To accomplish this, three experimental systems were used: a) staged seminiferous tubule segments, b) preputial testes from rat pups and c) Sertoli cell cultures. Using adult rats, and transillumination, seminiferous tubules were cut into four segments (pale, stages I-II; dark, stages III-VI; and dark zone, stages VII-VIII). All samples were incubated with or without 100 μM pervanadate (a membrane permeant tyrosine phosphatase inhibitor) for 15 min. @ 34.5°C to preserve/enhance phosphorylation patterns. Whole testis homogenates were made from 12- to 24-day-old rat pups and incubated with or without pervanadate. It is during this period of development that the TJ first forms. The third approach was Sertoli cell cultures grown both on plastic and permeable inserts in bicameral chambers. Protein samples were run on SDS-PAGE gels and immunoblotted using anti-phosphotyrosine monoclonal antibody (Upstate Biotech). Blots were stripped and reblotted using anti-actin antibody (gift of J.L. Lessard) to control for load. A comparison of the four tubule segments showed differences in multiple phosphoprotein bands, including 215, 175, and 130 kDa bands. The developmental study showed strong, changing phosphorylation staining in several bands, including the 175 kD band. The Sertoli cell cultures also had similar bands including a strong band at 215 kD and a weak 175 kD band in both the standard and bicameral chambers. These studies suggest that the phosphorylation status of cellular proteins including putative tight junction proteins varies with the stage of the seminiferous cycle, the development of the tight junction, and Sertoli cell culture conditions.

SERTOLIN IS A NOVEL SERTOLI CELL PRODUCT: ITS cDNA CLONING, TISSUE DISTRIBUTION, AND REGULATION. D. Moxley*, M. Mcgloin*, J. Griml, B. Stitzenberg, W.M. Lee**, and C.Y. Cheng. Population Council, Center for Biomedical Research, 3200 York Avenue, New York, NY 10021; Department of Zoology, University of Hong Kong, Hong Kong, and Institute of Pharmacology and Pharmacognosy, University of Rome, Italy.

Throughout spermatogenesis, there are extensive interactions between Sertoli (SC) and germ cell (GC) in the seminiferous epithelium largely due to the migration of developing germ cells from the basal lamina to the adluminal compartment. To identify molecules that are likely to be involved in SC-GC interactions, RNA differential display technique was utilized using RNAs isolated from primary cultures of SC, GC, and SC-GC. Briefly, an oligo (dT)3CA was used as the primer to reverse transcribe RNAs into cDNAs. Thereafter, PCR was performed using a random primer of 5'-ACCATGCCGCCCCGAGGAGCTG-3' (sense, nucleotides 1-30) and 5'-GSGTACCCGCTGCTCAA-3' (antisense, nucleotides 199-218) for RT-PCR, sertolin mRNA was detected in the adult rat brain, kidney, spleen, liver, lung, ovary, epididymis, testis, and innominate SC, but not in GC, heart, thyroid, thymus, or uterus. Furthermore, the steady-state mRNA level of sertolin increased by as much as 15-fold from 20 to 60 days of age during maturation suggesting its likely involvement in spermatogenesis. Moreover, sertolin mRNA expression was shown to be significantly stimulated by interleukin-1β (1-10 units/ml) but not by basic fibroblast growth factor (150 ng/ml) or interferon-γ (100 units/ml). Since sertolin is known to be a GC product and possibly released by GC, it is very likely that sertolin expression may in fact be regulated by GC. Hence, work is now ongoing to examine the effects of GC on SC sertolin expression in vitro. In addition, we are in the process of cloning the full-length sertolin cDNA from SC cDNA expression library constructed in an Uni-ZAP™-m directional vector (Stratagene). In summary, we have identified a novel SC gene designated sertolin. Results of this study show that sertolin is likely to be a novel marker in studying SC-GC interactions in vitro. Since SC and GC are the two main cell types present in the testis, we are using available pituitary cell lines. Northern blot analysis showed sertolin expression in the β cell line, a differentiated gonadotrope, but not in the α cell line, a less differentiated gonadotrope. CRES was also not expressed in the somatotrope/lactotrope GH3 cell line. Double immunofluorescence analysis of mouse pituitary tissue showed that CRES protein co-localized with LHβ, confirming that CRES is expressed in anterior pituitary gonadotropes. Western blot analysis of β gonadotrope cell lysate and conditioned media showed that CRES protein was secreted by the β cells. Interestingly, in contrast to the 19 and 14 kDa CRES proteins in the tests and epididymis, 17 and 12 kDa CRES proteins were present in the β cells. N-glycanase treatment of tests and epididymal lysates and β cell conditioned media showed that the higher molecular weight proteins (19 and 17 kDa) are the result of N-linked glycosylation. The highly restricted expression of the CRES gene to the tests, epididymis, and anterior pituitary gonadotropins suggests that CRES may be involved in several aspects of male reproduction.
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Abstract withdrawn.

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FAS/FAS LIGAND INDUCTION OF APOPTOSIS: A POSSIBLE MECHANISM OF HORMONE-DEPENDENT LEYDIG CELL ATTENUATION F.C. Griffin, J.S. Hushen, P. Barbour and D.F. Cameron, University of South Florida College of Medicine, Tampa, FL 33612

Fas and FasL proteins (TNF family) are involved in CTL-mediated cytotoxicity and down-regulation of the immune response by inducing suicidal apoptosis of activated lymphocytes. Although Fas and FasL have been detected in rat testes, their cellular localization is not well defined. Death of Leydig cells by hormone (LH) depletion was studied in vivo (hypophysectomized rats) and in vitro (primary cell cultures with and without hCG). Apoptosis was determined by TUNEL analysis; Fas and FasL was detected by Western blot analysis and immunohistochemistry. FasL appeared to be constitutively expressed on Leydig cells in normal testes and in fresh isolates. Fas, however, was not apparent in either freshly isolated cells or in Leydig cells of normal testes. When cultured for 48 h in non-supportive medium without hormone, Western blot analysis showed de novo expression of the Fas protein.

Results suggest that attrition of Leydig cells following LH depletion is by suicidal apoptosis, similar to that observed in activated T-lymphocytes, and involves the up regulation of Fas receptor and its subsequent binding by the already present FasL.

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DO FETAL LEYDIG CELLS DEGENERATE IN THE POSTNATAL RAT TESTIS? H.B.S. Aiyarayee and S.M.L.C. Mendis-Handagama. Department of Animal Science, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN.

Fetal Leydig cells (FLC) are present at birth but their destiny in the postnatal testis is still unknown. Therefore, we designed this study to understand the fate of the FLC in the postnatal rat testis. Sprague Dawley rats of 1,7,14,21,28,40,60 and 90 days were used. Their testes were either prepared for sterology (fixed in 2% gluteraldehyde in cacodylate and embedded in Epon-Araldite, w/v) or immunocytochemistry (Bouin’s fixed and embedded in paraffin, w/v). The presence of FLC and adult Leydig cells (ALC) in rats of 24-90 days of age was determined by 11β-HSD1 immunocytochemistry (FLC are 11β-HSD1-ve, ALC are 11β-HSD1+ve; additionally, the ratios of FLC:ALC numbers were obtained. FLC and ALC numbers in neonatal rat testes (1-21 days) were quantified by sterology; they were differentially identified on their morphology. FLC had euchromatic nuclei and lipid rich cytoplasm. ALC (observed from day 14 onwards) in 21 day testes had little or no cytoplasmic lipid in them. To quantify FLC and ALC in testes of 28-90 day old rats, first the total Leydig cell number per testis was determined by sterology and separated this value into FLC and ALC by using the number ratios for FLC:ALC obtained from the 11β-HSD1 immunocytochemical studies (mature ALC in the prepubertal-pubertal testes are also rich in cytoplasmic lipid, and therefore, this feature cannot be used to differentially identify these cells after day 21). The total Leydig cell number per testis was determined by immunocytochemistry (11β-HSD1+ve Leydig cells, i.e. FLC were continued to be present from birth to 90 days. Moreover, their number per testis did not change from birth to 90 days of age, although the ALC number per testis concomitantly decreased with age. This study convincingly demonstrated that FLC in rats do not degenerate in the postnatal testis, but continue to be present at least up to 90 days of age. (Supported by NSFBIN9409288, COE and Minkel Grants from UT).

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P53-DEFICIENCY SUPPRESSES GERM CELL APOPTOSIS AND STIMULATES CELLULAR PROLIFERATION IN VP10 Y. Lue, A.P. Sinha-Hikim, T.B. Rajavashisth*, C. Wang, W.E. Salameh and R.S. Swerdloff, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA.

The protein p53 is a key tumor suppressor, as evidenced by its frequent inactivation in human cancers. This study, using p53 +/- mice, addresses the functional role of p53 on germ cell homeostasis. Mice deficient in p53 exhibited an increased number in the numbers of both Brd U-labeled spermatocytes, but no apparent change in spermatogonial apoptosis, suggesting increased spermatogonial proliferation. This implies a lack of surveillance of various cell cycle checkpoints involving p53. The molecular basis of spermatogonial proliferation in p53 +/- mice is unknown. Apoptosis of spermatogonia in p53 +/- mice is associated with p53-dependent and p53-independent mechanisms. p53 is also expressed in prepubertal germ cells, which may illustrate the complexity of the p53 pathway in the testis.

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Differential Effects of Male Germ Cell Exposure to the Hypomethylating Drug, 5-Aza-Cytidine in Rat and Mouse Models. TE Deeken and JM Travieso. Dept of Pediatrics, Human Genetics and Pharmacology & Therapeutics, McGill University, and the Montreal Children’s Hospital Research Institute, Quebec, Canada.

DNA methylation patterns are established during gametogenesis and early embryogenesis, are essential for normal embryonic development, and have been implicated in gene regulation and genomic imprinting. We have shown previously that administration of the hypomethylating drug, 5-aza-cytidine (5-aza) to male rats for 11 weeks (exposes germ cells throughout spermatogenesis) interferes with normal male germ cell development and results in abnormal progeny outcome. Four weeks of treatment (exposes spermatids to mature sperm) had no effect on progeny outcome, and mature germ cell exposure (6 weeks of treatment) had an intermediate effect. Our recent studies suggest that these effects can be correlated with hypomethylation of germ cell DNA. There are limitations to the genetic and genomic imprinting studies which can be conducted in a rat model, so the goal of this pilot study was to confirm the progeny outcome effects of paternal administration of 5-aza in a mouse model. Male mice were treated for 3 weeks (exposes spermatids to mature sperm) and 7 weeks (exposes germ cells throughout spermatogenesis) with saline, 5-aza 40 (mg/kg, or 2-aza-5-aza-cytidine (2a) 0.8 mg/kg IP 3 times/week, then mated with normal females. Coeura sections were done at gestational day 18. Three weeks of treatment resulted in dose-dependent increases in infertility and preimplantation loss (C-3.75, L-7.78, H-40.93, 2a-3.03% preimplantation loss) and an increase in postimplantation loss with the 2a and analogue (C: 12.1, 2a: 25.8%). Seven weeks of treatment resulted in infertility, and those sperm exposed to 5-aza(L) were not able to fertilize eggs in in-vitro fertilization (C: 20.7%, L: 1.3% fertilization rate). We have confirmed that paternal 5-aza exposure in the mouse results in abnormal progeny, and, in contrast to the rat, there were effects with sperm exposure, suggesting that, in the mouse, normal patterns of DNA methylation may be necessary for the progression of spermatid into functional mature sperm. (Supported by MRC of Canada, and FSQ of Quebec).


Alterations in thyroid activity are frequently associated with changes in clinical and experimental male reproduction, but the exact mechanism by which thyroid hormones affect testicular development, maturation and function is still controversial. In the present study, we have investigated the effects of neonatal onset hypothyroidism on Leydig and Sertoli cell number and their functions by quantification of plasma & testicular interstitial fluid (TIF) testosterone and ABP concentration. Hypothyroidism was induced in neonates by adding 0.05% methimazole (MMI) in the drinking water of lactating mothers from the day of parturition till weaning (25 days postpartum) and then directly exposing the pups to drinking water containing 0.05% MMI for the remaining period. The rats were killed on day 30 and 60 postpartum. Hypothyroidism was confirmed by RIA of thyroid hormones (total and free T, T) along with TSH. Plasma FSH, LH and testosterone were assayed by RIA and ABP was quantified by radiometric method. Plasma thyroid hormones were decreased in hypothyroid rats and an opposite trend was observed in TSH, FSH & LH. Testicular weight, Sertoli and Leydig cell number, plasma and TIF testosterone and ABP were also decreased in hypothyroid rats of both groups. The present observations clearly shows that thyroid hormones are necessary for normal Leydig and Sertoli cell development, maturation and function.

Testicular Microlithiasis and Infertility Is Associated with Testicular Pathology. Jacques P Garnier, Keith Workman, Stephen F Shaaban, Univ. of North Carolina, Chapel Hill, NC 27522-7231

Introduction: Testicular Microlithiasis (TM) is an uncommon condition characterized by calcium deposits in the lumen of seminiferous tubules. These intratubular calcifications appear bright, 2-3 mm echogenic foci on testicular ultrasound. TM is a benign entity, yet can be associated with significant testicular pathology. We sought to evaluate the severity of this rare condition and its impact on male health by describing our experience.

Materials and Methods: 30 individual testicles in 18 patients were diagnosed with TM by high frequency testicular ultrasound over a 3 year period at two separate institutions. There was a wide range of indications for testicular imaging, including orchidalgia, infertility, and testicular masses. Pathologic specimens were available in 11 patients.

Results: Mean age at presentation was 28 years (range 8-52). Fifteen of 18 patients (83%) had bilateral TM. Five patients (28%) were infertile (4 secondary to hypergonadotropic testicular failure) with a mean FSH=46; range 24-65. Six patients (33%) had associated testicular malignancies (5 seminomas and 1 mixed germ cell tumor). Two patients presented with unilateral necrosis of the testicle due to spermatic cord torsion, and a third patient had an associated appendix testis torsion. Another 2 patients had associated varicoceles. We also describe previously unreported associations of TM and neurofibromatosis in one patient, and acquired immunodeficiency syndrome (AIDS) in another patient. Ultrasound follow up was available in 9 patients (50%), with a mean follow up of 32.1 months (range 1-96 months). Clinical follow up was available in 10 patients (56%), with a mean follow up of 37.8 months (range 1-108 months). One patient with bilateral TM was later found to have unilateral TM, and another patient with unilateral TM later developed bilateral TM. No patient with TM developed a testicular malignancy. One patient with previously documented TM developed spermatic cord torsion and testicular infarction.

Conclusions: TM is usually diagnosed by testicular ultrasound performed for various indications. We believe that TM is a benign condition that is found in testes associated with both malignant and non-malignant conditions, such as infertility. The association of TM and testicular malignancy mandates regular follow up with testicular ultrasound examinations.

Interaction Between Superoxide Dismutase (SOD) and cGMP on Nitric Oxide-Mediated Penile Erection in Rats. S.C. Sikka, G. Ruiz-Dreyfus & M. Rajasekar, and W. J. Hellstrom, Department of Urology, Tulane University School of Medicine, New Orleans, LA.

Nitric Oxide (NO) mediates penile erection via cGMP pathway leading to cavernosal smooth muscle relaxation. Reactive oxygen species such as superoxide anions (O2-), are known to interact with NO to generate toxic peroxynitrite radicals. Although SOD is a known scavenger of (O2-), its interactions with NO mediated penile erection pathway is unknown. We hypothesize that certain antioxidants such as SOD are likely to protect the NO from such interactions, thereby, prolonging the erectile response. In the present study, we investigated the erectile response in adult rats to intracavernosal injection of 8-bromo cyclic GMP (cGMP) in the presence and absence of SOD.

Adult male CD rats (N=6) were anesthetized with a combination of ketamine and xylazine. The left carotid artery and right penile crura were cannulated for the measurement of mean arterial pressure (MAP) and mean cavernosal pressure (MCP), respectively. cGMP (0, 10, or 30 µg in 0.1 ml volume) was injected into the left corpora cavernosa and a dose response was established. The changes in intracavernosal and systemic pressure were directly recorded into a computerized data acquisition program. SOD (100U) was injected intracavernosally and 10-minutes later the dose response to cGMP was evaluated. cGMP (0, 10, and 30 µg) produced a dose dependent increase in intracavernosal pressure (15, 56, and 80, mmHg), and in total duration of action (0, 190, 288 sec), respectively. A slight decrease in MAP (10 to 15% of baseline) was also observed. Intracavernosal injection of SOD prior to cGMP injection did not produce any significant change in MCP or MAP. However, cGMP (30 µg) administered 10-minutes after SOD injection showed a significant (50%) decrease in peak MCP, as well as in the total duration of response (308 sec vs. 288 sec, prior to SOD administration). There was no change in MAP with cGMP in presence of SOD.

This data suggests that SOD pre-treatment decreases the cGMP induced erectile response in rats instead of maintaining or prolonging it. Although SOD administration per se had no deleterious effects, its interactions with other mediators of penile erection warrant important consideration.
NITRIC OXIDE MEDIATED CYTOTOXICITY TO HUMAN CAVERNOSAL SMOOTH MUSCLE CELLS IN CULTURE

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Nitric Oxide (NO), a product of nitric oxide synthase (NOS) activity, is recognized as a central mediator of penile erection. Cavernosal smooth muscle relaxation is the main target of NO action. Human cavernosal smooth muscle cells (HCSMC) have been shown to express NOS in response to pro-inflammatory cytokines (e.g. IFN-γ). However, the toxicity of excessive NO produced under certain pathological conditions in the penis is not known. The present study was designed to evaluate whether NO induced cytotoxicity in isolated cavernosal cells in culture (monitored by DNA synthesis and ATP production) is via oxidative stress. Primary culture was initiated with penile explants from human corpora cavernosa and the monolayer cavernosal cells were plated on a 12 well tissue culture plate. At 70-80% confluence, the cells were incubated with sodium nitroprusside (SNP) (0.3-3 mM) for 16 hours. The DNA synthesis was evaluated by [3H] thymidine uptake. ATP levels (nM/10^6 cells) were measured by the luciferase method in a luminometer, whereas the total oxidative stress was monitored by measuring the level of 8-iso-PGF2α (pg/ml) using an ELISA kit. HCSMC exposed to SNP (1.6 mM) exhibited an 85% decrease in DNA synthesis. A significant decrease in the intracellular ATP levels (8.63 compared to 2.47 µg/ml/10^5 cells in controls) was observed. This was accompanied by a 100% increase in the levels of 8-iso-PGF2α (43.41 compared to 21.81 in controls) following SNP (0.8 mM) treatment.

These findings suggest that the NO released by SNP (>0.8 mM) exhibited a significant cytotoxicity to human cavernosal smooth muscle cells. Under the present conditions this NO induced cytotoxicity is probably mediated by increased oxidative stress to these cells.

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COMPARISON OF ERECTILE RESPONSES TO ANALOGS OF ANDRENOMEDULLIN IN THE CAT


Adrenomedullin (ADM) is a novel hypotensive peptide that shares structural similarity to calcium gene related peptide (CGRP). ADM has been identified in a number of organ systems, is present in human plasma, and may serve as a circulating hormone that regulates systemic arterial pressure. Champion, et al., have previously reported that ADM induces penile erection in the cat. The present study was undertaken to investigate the erectile responses to intracavernosal injections of analogs of ADM.

The peptides were injected directly into the corpus cavernosum and the reference drug combination (1.65 mg papaverine, 25 μg phenolamine and 0.5 μg PGE1) was injected intracavernously after each experiment as a control comparison. ADM (0.1-3 nmol) and ADM-(15-52) (0.1-3 nmol) caused penile erection in a dose-dependent manner. Intracavernosal injections of ADM-(22-52) (0.1-30 nmol) and ADM-(40-52) (0.1-30 nmol) did not alter cavernosum pressure and penile length. The maximal increase in penile length was comparable to the control with regard to ADM and ADM-(15-52). The erectile responses to ADM and ADM-(5-52) were similar, and the maximum effect on intracavernosal pressure was 75% of the control. A decrease in systemic blood pressure was observed after the administration of ADM (1 and 3 nmol), ADM-(15-52) (1 and 3 nmol) and the triple drug combination. L-NAME, a nitric oxide synthase inhibitor, and the KATP channel antagonist, U-73343A did not alter the erectile responses to ADM and ADM-(15-52).

The present study concludes that ADM and ADM-(15-52) produce penile erection when injected intracavernously, while ADM-(22-52) and ADM-(40-52) had no effect on penile intracorporal pressure. These data suggest that the erectile responses elicited by ADM and ADM-(15-52) are not mediated by nitric oxide or the opening of KATP channels. These data suggest the existence of alternative pathways to stimulate erections and support the clinical investigation of ADM and ADM-(15-52) to induce penile erection via intracavernous injection.

[TYR'-]NOCICEPTIN AND NOCICEPTIN HAVE SIMILAR NALOXONE-SENSITIVE ERECTILE ACTIVITY IN THE CAT

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The heptadecapeptide nociceptin, also known as Orphanin FQ, is a newly discovered endogenous ligand for the opioid-like G-protein coupled receptor, ORL1. We have recently shown that nociceptin has potent erectile activity in the cat. This study was undertaken to investigate the ability of intracavernosal injections of analogs of nociceptin and traditional opioid peptides to induce feline erections.

A 30-gauge needle was placed into the right corpus to permit administration of the peptide into the penis. The response was characterized by changes in intracavernosal pressure, duration of the maximal pressure, total duration of the drug effect, change in penile length, and alterations to the systemic arterial blood pressure. Of the reference drug combination (1.65 mg papaverine, 25 μg phenolamine and 0.5 μg PGE1), was injected intracavernously after each experiment for control comparison.

Intracavernosal injection of nociceptin and [TYR']-nociceptin in doses of 0.3-30 nmol induced dose-related increases in cavernosum pressure and penile length. Responses to [TYR']-nociceptin and nociceptin were rapid in onset and similar in duration. The duration of the responses to [TYR']-nociceptin and nociceptin was shorter than the responses to the triple drug therapy and the decreases in systemic arterial pressure were significantly less than with the triple-drug therapy. In contrast to responses to [TYR']-nociceptin and nociceptin, nociceptin-(2-17), nociceptin-(1-11), nociceptin-(7-17), β-endorphin, and dynorphin A did not induce increases in cavernosum pressure or penile length. Met-enkephalin increased cavernosum pressure and induced modest increases in cavernosum pressure at the higher doses studied, but was significantly less potent than [TYR']-nociceptin and nociceptin. Erectile responses to [TYR']-nociceptin and nociceptin were not blocked by the opioid receptor antagonist naloxone while responses to met-enkephalin were significantly reduced.

Results of this study in support the hypothesis that [TYR']-nociceptin and nociceptin have similar, naloxone-insensitive erectile activity in the cat. Moreover, the results demonstrate that the erectile activity is dependent upon the presence of 17 amino acids in the nociceptin sequence.

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PENILE ERECTION INDUCED BY PANAX NOTOGINSENG IN DOGS
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Panax notoginseng is an effective component extracted from traditional Chinese medicine. In order to explore the mechanism of PNS on penile erection, we studied the effects of PNS on penile erection by local PNS injection along with electrical stimulation in vivo. The results showed that: (1) Local injection of 1ml PNS (100mg/ml) in penis significantly increased the intracavernous pressure (ICP: 21.8±1.8mmHg), the duration of tumescence (DC: 18.5±2.7min) and the penile length (PL: 14±2.3cm), these effects were partially inhibited by injection of 1ml L-NAME (10mM/L), and the inhibition was partially reversed by injection of 1ml L-arginine (10mM/L). The results indicated that local injection of PNS in dog's penis induced erection through NO pathway. (2) Penile erection could be induced by electrical stimulation and 10mM/L -L-NAME could partially inhibit this effect. Among many different mechanism which involved in penile erection induced by stimulation of erectile nerves, the mechanism of NO pathway played an important role. However, other mechanism could not be excluded. The increment of ICP and PL induced by injection of PNS in penile corpus cavernosum were 41% and 47% of that by electrical stimulation. (3) The increment of ICP and PL induced by local injection of 1ml PNS (100mg/ml) in penis were 2 and 2.8 times of that induced by injection of 1ml Sodium nitroprusside (3mM/L), respectively. When the dosage of PNS was raised to 400mg/ml, only heart rate and blood pressure were slightly affected. In contrast, SNP at the concentration of 4-6mM showed severe hypotension.

In conclusion, NO release was increased by PNS from endothelium, which led to penile erection without affecting blood pressure. PNS might have a clinic value in the treatment of non-organic impotence.

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SPONTANEOUS EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) IN THE HYPOTHALAMUS AND FRONTAL CORTEX OF AGING RATS
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Excessive activation of the NMDA receptor (iNMDAR)/neuronal NOS (nNOS) cascade in the brain by excitatory amino acids may lead to neurodegenerative disorders through the release of cytotoxic levels of nitric oxide (NO) by pathological, nNOS neurons. In the hypothalamus, NO may impair the GnRH pulse secretion, either due to apoptosis of GnRH neurons or to feedback inhibition of nNOS affecting the physiological synthesis of NO (a positive modulator of GnRH-releasing). The aim of this study was to determine whether aging in the hypothalamus is associated with high NO synthesis, which is its source and whether this is organ-specific. Brown Norway male rats (N=5) at ages 1 ("immature"), 3 ("adult"), and 24 ("old") months, were used for measuring NMDARs in hypothalamic membranes by [3H]CGP binding. Another series of rats (N=9) was used for determining by western blot the contents of NMDAR, nNOS and iNOS in the hypothalamus, and only iNOS in the frontal cortex. NOS activity was measured in the hypothalamus by the arginine/citrulline assay. A significant decrease of NMDA analog binding was found in the hypothalamus from old rats as compared to adult (-66%) and immature animals (-57%), accompanied by a reduction in NMDAR content (-36% and -46%, respectively). In contrast, NOS activity in the hypothalamus was 68% higher in old rats as compared to the other two groups, but no significant differences were observed in nNOS content. However, hypothalamic iNOS increased nearly 4-fold in old rats, a process paralleled in the frontal cortex (3.6-fold). These results show that aging is associated with high NO synthesis in the hypothalamus and frontal cortex, independent of the NMDAR/nNOS cascade. We speculate that high cortical NO levels could lead to brain cell damage associated with aging.

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DO LOW DIHYDROTESTOSTERONE LEVELS CONTRIBUTE TO WASTING IN HIV-INFECTED MEN? S. Ayser*, L. Sinha-Hikim, R. Shen*, G. Beall*, M. Guerrero*, S. Basin. Division of Endocrinology, Charles Drew University and Harbor-UCLA Medical Center, Los Angeles, CA; and Karolinska Institute, Stockholm.

Low testosterone (T) levels, a common occurrence in HIV-infected men, are associated with wasting, muscle dysfunction, and poor disease outcome. Others have suggested that a defect in dihydrotestosterone (DHT) generation contributes to wasting in a subset of HIV-infected men. To determine if DHT levels correlate with weight loss or HIV-RNA copy number, independent of changes in T, we prospectively measured serum total and free T, DHT, LH and FSH, and SHBG levels in 150, HIV-infected, and 31 healthy men. Thirty-one percent of HIV-infected men had T levels <275 ng/dL; of these 81% were hypergonadotropic and 19% hypergonadotropic. Serum DHT levels were lower and T/DHT ratios (18.6 vs. 12.1; p<0.001) higher in HIV-infected men than controls. HIV+ men with normal T levels also had lower DHT (25 vs. 40 ng/dL; p<0.001) levels than controls. HIV-infected men who had lost >5 lb had lower total (48 vs. 80 ng/dL; p<0.001) and free T (49 vs. 60 pg/mL; p<0.003) than those who had not lost weight. Serum DHT levels did not differ between those who had lost weight and those who had not (19 vs. 24 ng/dL; p=NS). Serum T (r=0.2, p<0.025), but not DHT correlated with weight change and HIV-RNA copy number. Conclusions: DHT levels are lower in HIV-infected men than controls, but neither DHT nor T/DHT ratios correlate with weight loss or HIV-RNA copy number. The hypothesis that a defect in DHT generation contributes to wasting, independent of decreased T levels, remains to be proven. (Grant support: DK49296)

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CORTICOSTEROID CREAM PRE-TREATMENT DECREASES SEVERITY OF SKIN IRRITATION WITH ANDRODERM® IN HYPOGONADAL MEN. E. Rappaport, A. Haig and W. Albert SmithKline Beecham Pharmaceuticals, Collegeville, PA; T. Rallis, Dept. of Dermatology, Univ. of Utah, Health Sciences Ctr., Salt Lake City, UT.

Androderm® is a non-erectile testosterone transdermal delivery system available in the US and Europe for treatment of male hypogonadism. Most patients who use Androderm® experience some skin irritation at patch application sites. For some patients, skin irritation is sufficiently bothersome to discourage continued Androderm® use. To assess the effect of pre-treating patch application sites with emollient or steroid containing creams, we enrolled 30 hypogonadal men with bothersome skin reactions to Androderm® in a two-week clinical trial. During a 3 to 7 day screening period, skin irritation was assessed using a Modified Marzulli Irritation scale and patient diary cards and serum testosterone (T) and bioavailable testosterone (BT) concentrations were measured. Patients were randomly assigned to one of three pre-treatments: Cephil®, a hypoallergenic moisturizer (M); Cortaid®, 1% hydrocortisone cream (HC); or 0.1% tiamcinolone acetonide cream (TA). Skin irritation at patch-application sites and serum T and BT levels were re-assessed 1 and 2 weeks after initiation of pre-treatment. Improvements in skin irritation scores were observed in 4 of 10 (40%), 6 of 9 (67%), and 8 of 11 (73%) patients in the M, HC, and TA groups respectively. Patient diary data showed that TA markedly reduced redness and itching. M had little effect on these parameters and HC produced slight improvements compared to M. Mean baseline serum T levels were 609, 555, and 660 ng/dL and mean baseline BT levels were 312, 274, and 329 ng/dL in the M, HC, and TA groups respectively. Mean serum T and BT decreased in all groups; the greatest decreases in T and BT occurred in men receiving M pre-treatment. At the 2 week visit, serum T levels had decreased by 36%, 22%, and 8% and BT had decreased by 31%, 24%, and 11% in the M, HC, and TA groups respectively. One man in the M group who was not in the HC group and none in the TA group had serum T or BT levels below the lower limit of the assay reference range during the treatment period. The current study indicates that 1.) pre-treatment with 0.1% tiamcinolone cream decreases skin irritation at Androderm® application sites and 2.) in hypogonadal men using Androderm® for testosterone replacement, such pre-treatment does not have a clinically significant effect on testosterone absorption.
DIFFERENTIAL RATES OF CONVERSION OF TESTOSTERONE TO DIOXYDROTESTOSTERONE IN ANDROGENIC ALPHUS, ARAGELUS, ANGUILLUS, ANGOLOISE, G. PROKASSEN, H. A. C. VITOLLO, R. G. KENDT, N. BRATOS, AND B. W. WELLS. Mining University, Uniprasse, Poland, 11501 and State University of NY, Health Sciences Center, Stony Island, NY, 11501.

We report that all 11 alpha-esterases (AE) tested were able to hydrolyze testosterone (T) to dihydrotestosterone (DHT) in a dose-dependent manner without affecting T production. Each of these AE isomers, 5-K reductase type I (5-KR1) deficiency does not exhibit AA, suggesting the conversion of testosterone (T) to dihydrotestosterone (DHT) is a key mechanism in the promotion and progression of AA. We studied 49 healthy men, ages 17-35 years, 26 of them with AA (Hamlin Classification III to VII) and 25 controls, without family history of AA. Serum levels of T, DHT, Androstenedone (A), Androstenedione (AD), Estrone (E), Estradiol (E2), and Prostatic specific antigen (PSA) were measured by RIA.

Hormone  AA  Controls  p + Value
T (ng/dl ± SD) 60±153 64±117  <0.2
DHT (ng/dl ± SD) 62±14 58±18  <0.0001
A (ng/dl ± SD) 16±12 15±12  <0.01
Adiol (ng/dl ± SD) 10±13 9±17  <0.0001
E1 (ng/ml ± SD) 56±34 45±12  <0.05
E2 (ng/ml ± SD) 54±26 36±9  <0.02
PSA (ng/ml ± SD) 1.0±0.4 0.7±0.3  <0.02
T/DHT 9±6.09 17±7.33  <0.0001

Serum T was similar in both AA and control subjects. Serum DHT and Adiol G, however, were higher in subjects with AA. These results provide evidence of differential rates of conversion of 1 to DHT. In males with AA and strongly support the view that systemic DHT and peripheral conversion are important in the clinical expression of AA.

THE PESTICIDE LINDANE INHIBITS STEROIDOGENSES AND STEROIDOGENIC ACUTE REGULATORY (SAR) PROTEIN IN MA-10 LEYDIG CELLS. Lance P. Walsh* and Douglas M. Stocco*. Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas.

Lindane, the y-isomer of hexachlorocyclohexane (HCH) is widely used in agriculture, forestry and veterinary practice because of its insecticidal activity. It has been reported that y-HCH adversely affects male reproductive function. However, the actual mechanism by which y-HCH influences reproductive function remains unclear. Since Leydig cells play a pivotal role in male reproductive function through the production of the steroid hormone, testosterone, the mouse MA-10 Leydig tumor cell line was used as a model system to assess the potential effects of the HCH isomers on reproductive function. Testosterone production is stimulated by the action of gonadotropin on Leydig cells via the cAMP second messenger pathway. Therefore, MA-10 cells were stimulated with the cAMP analog, (Bc)2AMP, in the presence or absence of y-HCH or two other HCH isomers, a and R-HCH which are contaminants in commercial preparations of Lindane. Following stimulation we measured 1) steroid production, 2) the activity of the cholesterol side-chain cleavage (P450sc), 3) the activity of the 3a-hydroxysteroid dehydrogenase (3B-HSD), and 4) the effects of these compounds on protein synthesis. In addition, we measured the effects of these toxicants on the expression of the Steroidogenic Acute Regulatory protein (SAR), a protein which has been shown to be indispensable in the acute regulation of steroid hormone biosynthesis. SAR action results in an increased transfer of the substrate, cholesterol, from the outer to the inner mitochondrial membrane where it is metabolized to the first steroid synthesized, pregnenolone, an action which constitutes the rate limiting step in steroidogenesis. The results of these studies demonstrated that a, b, and y-HCH inhibited (Bc)2AMP stimulated pregnenolone production in MA-10 cells in a dose dependent manner without affecting total protein synthesis. In addition, P450sc and 3B-HSD activity and the expression of these enzymes were not affected by any of these compounds tested. In contrast, each of these HCH isomers had a profound effect on (Bc)2AMP stimulated expression of the SAR protein. Therefore, our results indicate that a, b, and y-HCH directly inhibit MA-10 mouse Leydig cell steroidogenesis by inhibiting STAR protein expression, thus impairs cholesterol transport to the inner mitochondrial membrane.

CYPROTERONE ACETATE (CYP) 5 mg/day PLUS TESTOSTERONE ENANTHATE (TE) 200 mg/week DOES NOT INDUCE A MORE PROFOUNED SPERM SUPPRESSION COMPARED TO CYP 5 mg/day PLUS TE 100 mg/week.

When administered in combination with TE 100 mg/week, higher CYP doses (100 and 50 mg/day) induced azoospermia in all subjects but caused a greater decrease of hematological parameters. With the administration of CYP 5 mg/day plus TE 100 mg/week, hematological parameters were not significantly affected but azoospermia was not consistently achieved. In this study we tested whether increasing the dose of TE administered in combination with CYP 5 mg/day would cause a more profound spermatogenic suppression. Thirteen normal men received CYP 5 mg/day plus TE 200 mg/week (CYP-5-200 m7) or plus TE 100 mg/week (CYP-5-100 m7) for 16 weeks. Seminal fluid analyses were performed every 2 weeks and blood samples were drawn every 4 weeks. RESULTS: The CYP-5-200 regimen did not induce a better suppression of spermatogenesis compared to the CYP-5-100 regimen. In week 16, sperm count was 0.1 ± 0.09 and 1.57 ± 0.79 M/ml in the CYP-5-100 and CYP-5-200 group, respectively.

azoospermia oligospermia oligospermia
CYP-5-200 0/7 6/7 (86%) 0/7 (14%)
CYP-5-100 2/5 (40%) 3/5 (60%) 0/5

No change of any blood chemistry, including HDL-cholesterol, could be detected with either regimen. No significant change of hematological parameters was found in either group. CONCLUSIONS: No improvement of sperm suppression could be found by increasing the dose of TE administered in combination with CYP. 2 Higher testosterone levels might have a direct stimulatory effect on spermatogenesis.

EFFECT OF TESTOSTERONE ENANTHATE ON STRUCTURE AND NUCLEAR DNA CONTENT OF RHESUS MONKEY PROSTATE.

T.S. Udayakumar, Alpesh Varghese, S.K. Das and M. Rajalakshmi, Departments of Reproductive Biology and Biotechnology, All India Institute of Medical Sciences, New Delhi – 110029

In order to involve men more extensively in family planning practices, thereby contributing to the control of population growth, funding agencies globally have been involved in developing male contraceptive regimens which are safe, reversible and effective. A major approach in this direction is the use of antispermogenic agents like progestogens and antiandrogens and GnRH analogues. In order to maintain peripheral levels of androgens physiologically, these agents need to be used in combination with androgens. But very little information is available on long-term use of androgens on prostate structure and function. In the present study, changes in morphology and cell cycle pattern of rhesus monkeys exposed to bimonthly injections of TE (50 mg i.m.) for 30 months is described. In TE-treated animals, increase in the size of prostate, acini and cell secretory activity were seen in cranial and caudal lobes of the prostate. Hyperplasia of cells in both lobes and hyperplasia in the cranial lobe, and increase in inter-acinar connective tissue were seen. Flowcytometric analysis of nuclear DNA content showed a shift from diploidy to the aneuploidy status in the TE-treated animals.
EFFECT OF TESTOSTERONE ENANTATE ON LIPID PROFILE IN LIVER FUNCTION TEST IN MONKEY
Alpana Tyagi and M. Rajalakshmii, Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi - 110029
One of the promising approaches to the development of a safe, effective, and reversible male hormone contraceptive is the use of exogenous sex steroid suppressive gonadotropin release thereby suppress spermatogenesis. But, very little information available of the side effects of long-term use androgens on metabolic and systemic functions. Hence, we have studied the effect of long-term administration of the commonly-used androstenedione enanthate (TE) on lipid profile, liver function parameters in rhesus monkeys. Correlates these effects with serum levels of testosterone. Adult rhesus monkeys were injected 50 mg of TE (i.m.) bi-monthly for two years in controls receiving only the vehicle. No significant change was observed in total cholesterol, LDL triglycerides levels in both groups. But HDL-C is decreased in treated monkeys. Changes were observed in plasma levels of alkaline phosphatase. Significant changes were observed, in SGOT and SGPT levels but within the normal range. It is noted that long-term administration of TE decreased HD levels in monkeys. The implication of such a decrement of the total protein in the liver may be discussed.

ROLES OF THE DISINTEGRIN DOMAINS OF THE SPERM PROTEINS FERTILIN α AND β IN FERTILIZATION
Fertilin is a mammalian sperm protein that is heterodimer of α and β subunits, both of which are members of a family of proteins known as ADAMs (A Disintegrin and a Metalloprotease domain) or MDCs (Metalloprotease Disintegrin-Cysteine-rich). A previous study has demonstrated that recombinant forms of the active extracellular domains of fertilin α and fertilin β bind to a human egg and inhibit sperm-egg membrane reaction. In this study, we made recombinant forms of fertilin α and fertilin β that include the disintegrin domains (αDCE and βDCE) or that are truncated so that they lack the disintegrin domains (αCE and βCE). Fertilin BDCE was able to inhibit sperm-egg binding, but fertilin BDE was relatively ineffective, indicating that the disintegrin domain of fertilin β is required for proper protein folding and/or for interactions with egg binding sites. Fertilin αDCE and αCE both inhibited sperm-egg binding, although fertilin βDCE was more effective. This indicates that the other domains of fertilin α are extracellular region (cysteine-rich and EGF-like repeat) have the ability to block sperm membrane reaction, but that the disintegrin domain enhances this ability, either by improving the efficiency of protein folding or by interacting with egg binding sites. Finally, since an anti-α antibody inhibits sperm binding (Almeida et al., 1995 Cell 81, 1095-1104) but not fertilin BDCE binding (Evans et al., 1997 Dev. Biol. 182, 79-93), fertilin αDCE or αCE might function as an α antigen integrin ligand. However, an anti-α antibody did not significantly inhibit the binding of either fertilin αDCE and αCE, suggesting that a different sperm ligand binds to an α antigen integrin on the egg surface.

ANGIOTENSIN II AND THE ACROSOME REACTION
S. Mueller*, U. Habemüller*, D. Drescher*, W.-B. Schill† and F.M. Koehn†, Center of Dermatology and Andrology, Justus Liebig University Giessen, Germany
Introduction: The renin-angiotensin-system is widely distributed in mammalian tissues. Until now the physiological functions on the male reproductive tract have not been clarified. Angiotensin II (AII) was found in Leydig cells in homogenates and within the epididymis. Therefore, AII plays a role in sertodgenesis and is important for sperm maturation. In earlier experiments we showed that AII induces the acrosome reaction (AR) significantly. The purpose of the present experiments was to investigate the second messenger pathways involved into the induction of AR by AII.
Materials and Methods: After glass wool filtration and two washing steps in HTFM (1% HSA), spermatozoa were treated with 100mM AII for 4h at 37°C. Since HSA may be contaminated with peptides and proteins, which cause degradation of AII, spermatozoa were washed in HTFM (without HSA) after incubation (3h, 37°C) and incubated with 100 nM AII for 15-60 min. The effect of AII on different second messenger systems was studied by preincubation of spermatozoa with an unspecific protease inhibitor H7 (10µM) or pertussis toxin (100ng/ml). The percentages of living acrosome reacted spermatozoa were determined by triple staining and compared to the corresponding values obtained after incubation with HTFM (control) and with 10 µM ionophore A23187 for 60 min after 3h capacitation (positive control).
Results: Compared to the negative control the percentages of acrosome reacted spermatozoa were higher after treatment with 100mM AII in HTFM (without HSA) for 45 min after 3h-capacitation (15.4% +/- 5.4% vs 6.4 +/- 2.8%, n=5). 60 min-incubation did not cause further increase of AR (15.0% +/- 5.4%, n=5). Treatment of spermatozoa with 100nM AII in the presence of HSA resulted lower rates of AR (9.8 +/- 2.5% vs 15.4 +/- 5.4%, n=5). Pretreatment with H7 showed nearly comparable values obtained to those of the negative control. Pertussis toxin did not influence the effect of AII on the AR. Conclusion: The induction of AR by AII is protein kinase- but not G-protein dependent. Peptidases and proteins that are present in the capacitation medium may inactivate AII.

MOLECULAR NATURE OF A SPERM ACROSOMAL ANTIGEN RECOGNIZED BY HS-13 MONOCLONAL ANTIBODY
T. Yoshiki and C.-Y. G. Lee*. Andrology Laboratory, Department of Obstetrics and Gynecology, The University of British Columbia, Vancouver, Canada.
Among the numerous monoclonal antibodies generated against human sperm antigens, HS-13 monoclonal antibody was shown to react with the antigen on the acrosome of spermatozoa from human, mouse and rat. In this study, HS-13 was used as the affinity ligand for the purification of the cognate antigen from human sperm by immunoadfinity chromatography. The purified cognate antigen from human sperm designated as HSAg-13 was found to be a protein with molecular weight of approximately 80 kDa on SDS-PAGE in the absence of reducing reagents. This monoclonal antibody was used as the probe to study the tissue distributions and developmental expression of the cognate antigen from human, mouse and rat. In this study, HS-13 was used as the affinity ligand for the purification of the cognate antigen from human sperm by immunoadfinity chromatography. The purified cognate antigen from human sperm designated as HSAg-13 was found to be a protein with molecular weight of approximately 80 kDa on SDS-PAGE in the presence of reducing reagents. This monoclonal antibody was used as the probe to study the tissue distributions and developmental expression of the cognate antigen from human sperm. Following calcium ionophore A23187 treatment, acrosome-reacted sperm showed a negative staining, suggesting the intra-acrosomal location of HSAg-13. The induced acrosome reaction resulted in a statistically significant decrease of antibody-stained acrosomal location of HSAg-13. The induced acrosome reaction resulted in a statistically significant decrease of antibody-stained acrosomal location of HSAg-13.
Acrosome reaction is a prerequisite for successful fertilization. Information on the inter and intrasperm variations in acrosome reaction results is not readily available. Our study examined the inter- and intrasperm reaction variation in assessing the acrosome reaction in cryopreserved sperm. Semen specimens were obtained from 15 healthy volunteers with proven fertility according to the World Health Organization criteria. The ejaculates were diluted after liquefaction (1:1, vol/vol) with TRIS-yolk buffer containing glycerol and then frozen in liquid nitrogen. The spontaneous acrosome reaction was assessed by fluorescein isothiocyanate conjugated peanut lectin. Sperm viability was analyzed with the average coefficient of variation (CV), and the intraobserver variability. The differences in reaction frequency between observers were analyzed with the average coefficient of variation (CV), and the intraobserver difference was -0.29% (P < 0.05; ICC = 0.98 at 95% confidence interval). We conclude that sperm which did not attach to OEC were of inferior quality as compared to those sperm which did attach to the OEC. This interaction may be valuable for isolating superior quality sperm to be used in clinical procedures. Funded by NIH HD 32851.

ATTACHMENT OF HUMAN SPERM TO OVOCYTE CELLS IN VITRO SELECTS FOR HIGHER QUALITY SPERM.
Ellington J., Wright R*, Joat L*, Schneider C*, Jones A*, & Evenson D*.

Human sperm attach to oviducal epithelial cells (OEC) grown on Matrigel in vitro. This study evaluated if attachment selects for any specific sperm quality type. Expt. 1 utilized Percoll washed sperm (1.5 X 10^7/ml) placed into coculture with follicular phase bovine OEC monolayers in HTF + HSA (n=7 ejaculates). Measurements included percent: viable sperm (eosin-nigrosin stained); sperm with normal membranes (hypo-osmotic swell test "HOS"); and motility. These measurements were compared after 2 hrs of culture for sperm which selectively did not attach to OEC in coculture versus sperm in medium alone as controls. In Tables, a and b differ at p<0.05 and data is expressed as mean (SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Viable</th>
<th>% Normal HOS</th>
<th>% Motile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not attached to OEC</td>
<td>66(5)*</td>
<td>63(4)*</td>
<td>69(5)*</td>
</tr>
<tr>
<td>Control media</td>
<td>65(4)*</td>
<td>62(6)*</td>
<td>69(5)*</td>
</tr>
</tbody>
</table>

In Expt. 2, the sperm chromatin structure assay (detecting susceptibility to DNA denaturation and strand breaks), was compared between sperm which did not attach versus those which did attach to OEC after 2 hrs of coculture (n=4 ejaculates).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Abnormal Chromatin (CV(%)</th>
<th>% Variability (%Mot.</th>
<th>% Immature Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Attached to OEC</td>
<td>12(5)</td>
<td>125(3)</td>
<td>4(0.3)*</td>
</tr>
<tr>
<td>Attached to OEC</td>
<td>2(5)</td>
<td>130(3)</td>
<td>8(0.3)*</td>
</tr>
</tbody>
</table>

In general, sperm which did not attach to OEC were of inferior quality as compared to those sperm which did attach to the OEC. This interaction may be valuable for isolating superior quality sperm to be used in clinical procedures.

SEMEN PH AND ITS RELATION TO SPERM FUNCTION
N. Virgi, S. Goldberg, H.M. Nagler. Department of Urology, Beth Israel Medical Center, New York, NY.

In order to determine if the routine measurement of pH in semen is warranted, semen analysis data on 152 nonazoospermic men referred to the Andrology Laboratory was retrospectively examined. Data included semen pH (determined by pH paper within 1 hr of ejaculation), sperm concentration and count, percent motile sperm, sperm viability as determined by supravital staining and hamster egg penetration assay score expressed as the average number of penetrations per ovum. Statistical analyses was performed with SAS. The Pearson correlation test was used to determine if there was a linear relationship between pH and the various semen variables.

The mean ± SD for pH was 8.4 ± 0.32, range = 7.8 - 9.2. There was no correlation between semen pH and sperm concentration, motility and viability. However, the pH showed only moderate correlation with the total motile sperm count (r = -0.18, P=0.02). There was no correlation between pH and the SPA score (r = -0.12, P=0.14).

Studies of fertile and infertile men have shown no relation of semen pH with the fertility status. We found no correlation between pH and other semen variables. This is in agreement with previous findings by others. Moreover, the lack of correlation between pH and the SPA indicates that sperm function in vitro may not be affected by semen pH. In our study, pH was considerably higher than the normal range established by the WHO (7.2 - 8.0). Although our study samples may not be completely representative of samples from normal fertile men, our findings indicate that the routine determination of semen pH may not be warranted. This is supported by our data in which 37% of the men who had normal count, motility and SPA score also had higher pH than recommended by the WHO.

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ATTACHMENT OF HUMAN SPERM TO OVOCYTE CELLS IN VITRO SELECTS FOR HIGHER QUALITY SPERM.

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SEMEN PH AND ITS RELATION TO SPERM FUNCTION

2023 Annual Meeting of the American Society of Andrology
MOTILITY ENHANCEMENT OF CANINE EPIDIDYMAL SPERM
Barbara Durrant, Kara Russ*, Sally Harper*, Thomas DiBerto*, Cathy King*, Cheryl Rosa* and Michael Kiburtz*

Epidydimal sperm represents a reservoir of genetic material which can be opportunistically salvaged at castration or necropsy. Although this resource has been exploited for the preservation of endangered species in "frozen zoos", poor post-thaw motilities limits its usefulness. There have been no reports describing the empirical derivation of appropriate cryopreservation techniques for epidydimal sperm of any carnivore except the domestic cat. In vitro capacitation and motility enhancement techniques have not been developed. In this series of experiments the domestic dog served as a model for exotic carnivores.

Sperm was extracted from minced cauda epidydimals following routine neutering for population control. Following suspension in BWW medium with 1% BSA, sperm was assessed for motility and % motility (MS) and was assessed by CASA (HTM-2030; Hamilton-Thom Research). The initial mean motility was more motile than 20 mM at T1, but not at T2. Although not significantly different in both treatment groups had significantly higher %MS than controls (p<.05). The motility and % analyses were repeated at 30 min (T1) and 60 min (T2) of incubation. To correct for differences between dogs, all results were expressed as % Initial MS (IMS) and % Initial L (IL). Experiment 1 compared the effects of 0, 5, 10, 20 and 40 mM CA on sperm from three donors. At T1 and T2 only 20 and 40 mM treatment groups had significantly higher %IMS than controls (p<.05). The 40 mM group was more motile than 20 mM at T1, but not at T2. Although not significantly different from the control group, there was a slight reduction in %IL at the two highest CA concentrations at T1 and T2. Experiment 2 compared CA (20 mM) with IBMX (250 µM) and DA (20 mM). Both CA and DA significantly reduced %IL at T1 compared to untreated controls, but the effect was not significant by T2. At three hours significantly increased %IMS at T1 and 2, with CA and DA having a greater effect than IBMX. These results indicate that CA and DA, each at 20 mM, can maintain increased motility in thawed epidydimal canine sperm for at least one hour without significant loss of viability. In both experiments, increased MS was due primarily to enhanced SOP.

These data suggest that motility enhancement of cryopreserved epidydimal sperm can be a useful adjunct to assisted reproduction in exotic carnivores.

Other spermatogenesis is required to travel a long distance in vivo to meet the oocyte at the fertilization site. One of the many explanations of male fertility is that sperm populations may be less capable of reaching the oocyte because of their unique inherent or acquired migratory potential.

There is no difference in the % motility, and motile count between ENH, PS and PureSperm® (PS) with respect to recovery (of motile sperm), motility (%), path (Vcl) and progressive velocity (V5) (um/sec) and hyperactivation (%). Pregnancy rates (PR) were also retrospectively examined for the period of January, 1997-March, 1997, and April, 1997- July, 1997 during which time the ENH and PS methods, respectively, were being used. Semen specimens (n = 25) obtained within 30 minutes of ejaculation were assessed by CASA (HTM-2030; Hamilton-Thom Research). The initial mean sperm count was 63.9 x 10^6 (range 1.5 to 145). One milliliter of semen was placed onto an Enhance gradient (1.5ml each; 80%/45%), and centrifuged at 300 g for 20 min. The pellet of good progressively motile sperm for IUI or ART, but PRs appear to be lower than PS with ENH.
LOCAL ANESTHETICS IMPAIR HUMAN SPERM MOTILITY
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Introduction: Local anesthetics have well described effects on a variety of signal and energy transduction systems. We analyzed human sperm in the presence of two local anesthetics to determine whether these agents could delineate metabolic and cell-signal determinants of sperm motility.

Materials and Methods: Sperm from a normal volunteer was diluted in PBS. CASA was performed in a control specimen and during exposure to lidocaine (4 and 8 mM) and bupivacaine (1 and 2 mM).

Results: Total motility (see figure) and sperm velocity showed dose-dependent inhibition with both local anesthetics. Tail beat frequency was not affected by either local anesthetic.

Discussion: We have shown that local anesthetics with different chemical profiles inhibit sperm motility but not beat frequency. The effects on motility are dose-dependent and proportional to anesthetic potency. Both local anesthetics inhibit voltage-gated ion channels; bupivacaine also impairs adrenergic receptor binding, lysophosphatidyl signalling, and mitochondrial respiration. These findings suggest that local anesthetics can serve as chemical probes of the cellular dynamics underlying human sperm motility.

THE EFFECTS OF REACTIVE OXYGEN SPECIES ON HUMAN SPERM METABOLISM AND MOTILITY: IMPLICATIONS FOR THE ROLE OF INTRAMITOCHONDRIAL LDH-C4
J.S. Armstrong*, M. Raynak-raz, W. Heffernan and S. C. Sikka. Department of Urology, Tulane University Medical Center, New Orleans, LA

Sperm motility is a prerequisite for normal fertilization. Reactive oxygen species (ROS) are known to adversely affect sperm motility and damage sperm membranes. In this study we have characterized the ROS responsible for inhibition of sperm motility, and identified possible molecular targets of sperm energy metabolism affected by ROS.

Human sperm are routinely prepared in HAM's F-10 nutrient media and exposed to (a) H2O2 (500µM) + X0 (0.1UI/ml) and (b) FMA (2µg/ml) stimulated "buffy coat" leukocytes (3.5 x 10³/ml) in the presence and absence of SOD (100U/ml), catalase (200KU/ml) and mannitol (40mM) (GAPDH inhibitor) and KCN (1mM) (cytochrome oxidase inhibitor) were used to clarify important pathways of sperm energy metabolism and help identify possible molecular targets of ROS action. Sperm motility (%), viability (%), HOST (%), ATP (nmol/10⁶ sperm), lactate (mg/10⁶ sperm) and B-iso-PGF, (picograms/ml/10⁶ sperm) were evaluated.

Spermatozoa treated with ROS exhibited a concentration and time dependent decrease in ATP levels and motility. Neither SOD nor mannitol affected this inhibition but catalase reversed it. Viability, HOST and B-iso-PGF, levels in spermatozoa treated with ROS were not significantly different from control values. FMA stimulated "buffy coat" leukocytes also inhibited sperm motility that could be protected with catalase but not with SOD. Treatment of sperm with either ROS, KCN, IOA or combination of KCN/IOA inhibited sperm motility and ATP levels significantly. This could be reversed with pyruvate (20mM) indicating sperm ATP generation by the citric acid cycle independent of oxidative phosphorylation. Lactate levels decreased significantly following inhibition of glycolysis with IOA and oxidative phosphorylation with KCN. Lactate, however, was not affected by ROS.

These studies suggest that ROS mediated inhibition of sperm motility is independent of loss of cell viability and lipid peroxidation. It seems likely that the decrease in ATP following exposure to ROS is related to inhibition of ATPase-synthase activity as ROS even at high doses, did not appear to affect glycolytic flux or electron transport. Pyruvate protection of motility suggests redundancy of oxidative phosphorylation for sperm motility.

The unique location of intramitochondrial LDH-C4 and its role in pyruvate/lactate metabolism may represent an evolutionary safeguard ensuring sperm survival in times of metabolic stress.

ENHANCEMENT OF SPERM MOTILITY BY NITRIC OXIDE IN FATAHEAD MINNOWS, PIMEPHELAS PROMELAS
M. M. Creech*, R.W. Atherton, E. Arnold* and S. Bohle*, Departments of Zoology/Physiology and Chemistry, University of Wyoming, Laramie WY.

The effect of nitric oxide (NO) on sperm motility was examined in the Fathead Minnow, Pimephales promelas, using computer assisted sperm analysis (CASA). The dual nature of NO can be seen in its dose dependent effects on sperm. Ten millimolar sodium nitroprusside (SNP), an NO donor, decreased percent motile sperm and inhibited velocity parameters; VCL, VSL and VAP, while one micromolar SNP enhanced percent motility and increased velocity. Evidence that NO exists in the fertilization environment of Fathead minnows was provided by electron spin resonance (ESR). A characteristic NO signal was produced by buffer surrounding oocytes within a critical five minute period following laying. Tests of "egg water" after five minutes produced no signal indicative of rapid transformation of the NO radical. Nitric oxide synthase (NOS) was histochemically localized at the micropyle of mature unfertilized eggs and inhibitors of NOS blocked histochemical staining. This time dependent release of NO by eggs and localization of the enzyme to the site of sperm entry, coupled with the motility enhancing effect of micromolar concentrations of NO on sperm suggests an active role for nitric oxide in fertilization.

EVALUATION OF THE HEPARIN-INDUCED SPERM CHROMATIN INSTABILITY, AND ITS CORRELATES WITH THE SPERM PENETRATION ASSAY AND SPERM MOTILITY. CONCENTRATION, HOS AND MORPHOLOGY. B.R. Emery, C.M. Peterson*, D.T. Carroll, University of Utah School of Medicine, Salt Lake City, Utah.

Heparin has been shown to induce the unraveling of super-coiled chromatin within some spermatozoa. In this study we have evaluated the relationship between the frequency of heparin-induced chromatin decondensation and the characteristics of the semen sample obtained by semen analysis and the sperm penetration assay (SPA). Semen samples were analyzed from 34 men, including infertility patients and semen donors of known fertility. Semen samples were washed and incubated with 50 USP of heparin for one hour, then stained and the percentage of sperm exhibiting decondensation was determined. A portion of the sample was also analyzed by standard semen analysis techniques, including the HOS assay, and the SPA. No significant correlation was seen between the decondensation rate and ROS rate, sperm motility, concentration, and the percentage of morphologically normal heads. A nonsignificant inverse correlation (r=-0.21, p=0.11) was observed between the decondensation rate and the SPA rate. The mean decondensation rate was 3.7±0.6 (Mean ± SE) for donors of known fertility, significantly different than 7.8±1.5 (p<0.01) patients of general infertility. Patients with a decreased penetration rate had a significantly higher rate of decondensation than that of donors (21.7±1.8, p<0.001). These data indicate that the level of decondensation following heparin exposure may be related to factors that affect sperm penetration ability.
ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) ON HUMAN SPERMATOZOA.

It has been reported that nitric oxide (NO), a ubiquitous free radical gas produced by the nitric oxide synthases (NOS), may be involved in normal and abnormal sperm function. The objectives of this study were to determine if human spermatozoa express eNOS and whether the expression of eNOS on spermatozoa correlates with sperm motility. Semen samples (n=12) were obtained from non-azoospermic men presenting for infertility evaluation at our institution. Samples were fractionated into high, intermediate and low density subpopulations on discontinuous Percoll gradients in order to examine the correlation between eNOS staining on spermatozoa and sperm motility. eNOS protein was detected using a previously characterized eNOS monoclonal antibody (Zini et al, Biol Reprod 55: 935-941, 1996). Control sections were incubated with preabsorbed antibody or mouse IgG. eNOS was primarily detected on the head and midpiece regions of abnormally shaped spermatozoa. A significant negative correlation was observed between the percentage of sperm with eNOS immunostaining and the percentage of motile sperm (r = -0.46, p<0.05). The preferential localization of eNOS on abnormally shaped spermatozoa suggests that sperm-bound eNOS is unlikely to be involved in normal sperm function. Alternatively, sperm eNOS may be responsible for the generation of cytotoxic oxidants.

SEmen Variables as Determinants of Sperm Separation

EVALUATION OF A MULTI-LABORATORY, SEVEN YEAR,
SEmen ANALYSIS QUALITY CONTROL PROGRAM. D. Carmill, C. Thorp*, T. Carnell, University of Utah School of Medicine, Salt Lake City, UT.

The semen analysis is the main diagnostic test reported by an Andrology laboratory. It is therefore important that the performance of each technician be closely and continuously monitored. Our Andrology laboratory has developed a semen analysis quality control program, which has been performed in conjunction with four other Andrology laboratories for the past seven years. Complete semen analyses are performed monthly on cryopreserved semen lots. Additionally, specific parameters of the semen analysis are studied in multiple lots on a quarterly basis. Performance is evaluated for progressive sperm motility, viability, hypoosmolality, quantitative morphology and sperm count. Results are analyzed, standard deviations calculated and subsequently monitored for individuals performing outside of acceptable limits. Competence is determined, and corrective action or retraining is given based on the QC results. Analysis of the quality control data indicates that these monitoring and retraining techniques have allowed the acceptable range of performance to be gradually changed from two standard deviations to one standard deviation over time, without increasing the number of technicians out of the maximum allowable range. The lowest coefficients of variance are normal sperm head morphology (0.04-0.27) and sperm tail morphology (0.05-0.18). The widest range of variance occurs with specific classes of abnormal sperm head morphology such as immature and amorphous shapes. Trends have been noticed with individual technicians and continuing education has been given to prevent divergence from standards. This is an invaluable part of the quality control program. These data suggest that a carefully monitored quality control program is useful in maintaining and evaluating laboratory technician proficiency.
THE HAMSTER SPERM ANTIGEN P26h IS A GLYCO­PHOSPHATIDYL-INOSITOL ANCHORED PROTEIN

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We have previously identified a hamster sperm protein, P26h, that is proposed to be involved in the cascade of events governing the interaction between spermatozoa and the egg's zona pellucida. In this study we investigated the mechanism of P26h accumulation on hamster spermatozoa during epididymal maturation. Immunocytochemical studies using an anti-P26h antiseraum showed an accumulation, from the caput to the cauda epididymal segment, of this protein on spermatozoa. P26h was exclusively localized on the acrosomal cap of the mature spermatozoa. In order to document the anchoring mechanism of P26h, cauda epididymal spermatozoa were exposed to different treatments. Exposures to high-salt buffered solutions were unable to remove P26h from the surface of intact cauda epididymal spermatozoa. P26h was efficiently released when live spermatozoa were treated with a phospholipase C specific to phosphatidylinositol. The amount of P26h released from the sperm surface was proportional to the concentration of phospholipase C used. In contrast, all the P26h remained associated to the sperm surface following treatment with trypsin. Proteasomes, present in the epididymal fluid have been proposed to be involved in the transfer of GPL anchored sperm proteins. Our original hypotheses were therefore confirmed using ultrasensitive and specific antisera to phosphatidylinositol. Western blot analysis confirmed the presence of P26h on the surface of hamster spermatozoa. In contrast, all the P26h remained associated to the sperm surface following treatment with trypsin. This result suggests the presence of a cell to cell transfer of a phosphatidylinositol-anchored protein which would be part of the epididymal maturation process of the hamster spermatozoa. Supported by MRC Canada.
Cryopreservation and recovery of motile, aspired human sperm within biopsy capsules from hamster eggs.

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Problems encountered with the use of testicular or epididymal aspirated sperm for IVF/ICS1 include: a) limited numbers of sperm retrieved and b) recovery of viable sperm after cryopreservation due to low motility and tissue contamination. To address this second limitation, we evaluated a novel cryo-preservation approach in which 'ICS1-ready' motile sperm are placed and frozen within biopsy capsules from emptied hamster zona pelliculosa (Cohen, J. et al., Hum. Reprod. In press)

With IRB consent, ejaculated (n=3 patients), epididymal (n=3 patients) and testicular (n=3 patients) sperm were recovered for viability by motility and vital stain (carbohydr Fluorescein and propidium iodide), and each sample aliquoted and cryopreserved by two methods: 1) sperm were frozen and thawed in TST-yolk-glycerol buffer using conventional methods and 2) in parallel, 35-50 motile sperm from each sample were microinjected into hamster zona capsules. Zona-encapsulated sperm were frozen by microdrop transfer into medium cryopreserved sperm: ejaculated-7% motile, 29% viable; epididymal-capsules from emptied hamster zona pellucidae (Cohen, second limitation, we evaluated a novel cryo-preservation approach in conventional methods and 2) in parallel, 35-50 motile sperm from each sample aliquoted and cryopreserved by two methods: (Supported by a UC-Los Alamos National Laboratory Directed Research and Development Grant to MAM).

In this study, we assessed the effects of osmotic stress (hyp- and hyperosmolarity) on sperm from both N and T cats by evaluating the proportion of intact plasma membranes (IPM). Electorejacularated (2 males/group; 2 ejaculates/male) were diluted 1:1 in Ham's F10 + 25 mM HEPEs + 5% FCS (300 mOsm), washed by centrifugation, and the resulting pellets were resuspended and maintained at 22°C. Hypoosmotic solutions (<35,75 and 150 mOsm) were prepared by diluting Ham’s F10 with reagent grade water, while hyperosmotic solutions (500 and 1200 mOsm) were prepared by diluting Ham’s F10 to 10X solution of Ham’s F10. Ten µl of sperm suspension were added to 500 µl of the respective test solutions, equilibrated (10 min, 25°C) and evaluated for IPM following staining with SYBR-14 (intact) and propidium iodide (non-intact). Sperm from N males exhibited a precipitous decline in IPM following exposure to hypoosmotic solutions (300 mOsm, 94%; 150 mOsm, 94%; 75 mOsm, 75%; 35 mOsm, 14%; 0 mOsm, 0%). However, sperm from T males exhibited a more dramatic decline (300 mOsm, 100%; 150 mOsm, 53%; 75 mOsm, 20%; 35 mOsm, 4%; 0 mOsm, 0%). In contrast, sperm from both populations were resilient (p>0.05) to hyperosmotic stresses and maintained high proportions of IPM at 600 mOsm (75-97%) and 1200 mOsm (79-94%). Critical toxicity (osmolarity at which 50% of the cells swell and lyse) was 58 mOsm for N cats and 142 mOsm for T cats. These results indicate that sperm from T males are more susceptible to osmotic stress than their N counterparts, which in turn, may arise from differences in membrane composition or flexibility. (Smithsonian Institution Scholarly Studies Program and Women’s Committee).

Increased susceptibility to sperm membrane damage due to osmotic stress in teratospermic cats. B.S. Pukazhenthi, A.M. Donoghue§, D.E. Wild§, E.E. Nobile§ and J.G. Howard. National Zoological Park and Conservation & Research Center, Smithsonian Institution, Washington, DC 20008; and Spermplasm and Gamele Physiology Laboratory, ARS, USDA, Beltsville, MD 20705.

We recently reported that sperm from teratospermic (T; >60% morphologically abnormal sperm) domestic cats are more susceptible to both cooling and freeze/thaw induced sperm membrane damage than normospermic (N; >60% normal sperm) cats (J. Androl. Suppl., 1997). To develop an optimal sperm cryopreservation protocol for cats, we have begun assessing factors affecting sperm plasma membrane integrity. In this study, we assessed the effects of osmotic stress (Sperm- and hypoosmolarity) on sperm from both N and T cats by evaluating the proportion of intact plasma membranes (IPM). Electorejacularated (2 males group; 2 ejaculates/male) were diluted 1:1 in Ham’s F10 + 25 mM HEPES + 5% FCS (300 mOsm), washed by centrifugation, and the resulting pellets were resuspended and maintained at 22°C. Hypoosmotic solutions (0, 35,75 and 150 mOsm) were prepared by diluting Ham’s F10 with reagent grade water, while hyperosmotic solutions (500 and 1200 mOsm) were prepared by diluting Ham’s F10 to 10X solution of Ham’s F10. Ten µl of sperm suspension were added to 500 µl of the respective test solutions, equilibrated (10 min, 25°C) and evaluated for IPM following staining with dyes.

Comparison of creatine kinase activity between fresh and cryopreserved sperm from normozoospermic and subfertile men.

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Pregnancy rates are poor with cryopreserved spermatozoa compared with fresh sperm, regardless of male fertility status. Sublethal damage to the plasma membrane occurs during the freeze-thaw process, resulting in poor sperm function and motility characteristics. In fresh spermatozoa, elevated creatine kinase (CK) activity is related to an abnormal cytoplasm level and morphological defects in sperm. This study determined whether sperm cell damage and changes in functional characteristics that occur during the freeze-thaw process are related to sperm mid-piece swelling and a decrease in mid-piece swelling (P <0.0009). This strong correlation between activity using a kit (Sigma Diagnostics). Results are expressed as median and interquartile range. The average spermatozoal creatine kinase (CK) level indicates sperm maturity and correlates with spermatozoa fertilizing potential. Excessive cytoplasmic CK near the mid-piece region is linked to sperm immaturity. Our study assessed the association between specific sperm morphological abnormalities and the sperm CK activity in subfertile men. Semen specimens were collected from 66 men who were subfertile according to the World Health Organization (WHO) criteria. Characteristics of liquefied semen were analyzed using a computer-assisted semen analyzer. Seminal smears were stained with a Giemsa stain (Diff-Quick) and morphological features were scored according to the WHO method. Creactine kinase activity was determined with a CK kit (Sigma Diagnostics) by suspending a semen aliquot in imidazole buffer at pH 7.0 and measuring the change in absorbance of NADH at 340 nm. Results were expressed as units/10⁶ sperm. and the relationship between CK activity and sperm morphological abnormalities was analyzed. The average CK activity was 0.46 ± 0.08 units/10⁶ sperm in the 66 samples. The CK level was significantly lower when the percentage of normal sperm form was high (P <0.001). A high CK level significantly correlated with an increase in mid-piece swelling (P <0.0009). This strong correlation between sperm mid-piece swelling and CK suggests that these abnormalities may be related to defects in cytoplasmic extrusion or in mitochondrial function, which may impair spermatozoal motility.

Correlation between sperm mid-piece abnormalities and creatine kinase content in subfertile men.

R.K. Sharma, D. Garlak, A.J. Thomas and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland OH.

The spermatozoal creatine kinase (CK) level indicates sperm maturity and correlates with spermatozoa fertilizing potential. Excessive cytoplasmic CK near the mid-piece region is linked to sperm immaturity. Our study assessed the association between specific sperm morphological abnormalities and the sperm CK activity in subfertile men. Semen specimens were collected from 66 men who were subfertile according to the World Health Organization (WHO) criteria. Characteristics of liquefied semen were analyzed using a computer-assisted semen analyzer. Seminal smears were stained with a Giemsa stain (Diff-Quick) and morphological features were scored according to the WHO method. Excessive cytoplasmic CK near the mid-piece region is linked to sperm immaturity. Our study assessed the association between specific sperm morphological abnormalities and the sperm CK activity in subfertile men. Semen specimens were collected from 66 men who were subfertile according to the World Health Organization (WHO) criteria. Characteristics of liquefied semen were analyzed using a computer-assisted semen analyzer. Seminal smears were stained with a Giemsa stain (Diff-Quick) and morphological features were scored according to the WHO method. Creactine kinase activity was determined with a CK kit (Sigma Diagnostics) by suspending a semen aliquot in imidazole buffer at pH 7.0 and measuring the change in absorbance of NADH at 340 nm. Results were expressed as units/10⁶ sperm. and the relationship between CK activity and sperm morphological abnormalities was analyzed. The average CK activity was 0.46 ± 0.08 units/10⁶ sperm in the 66 samples. The CK level was significantly lower when the percentage of normal sperm form was high (P <0.001). A high CK level significantly correlated with an increase in mid-piece swelling (P <0.0009). This strong correlation between sperm mid-piece swelling and CK suggests that these abnormalities may be related to defects in cytoplasmic extrusion or in mitochondrial function, which may impair spermatozoal motility.
Y CHROMOSOME DELETIONS IN AZOOSPERMIC AND SEVERELY OLIGOSPERMIC MEN UNDERGOING TESTICULAR SPERM EXTRACTION (TESE) AND INTRACYTOPLASMIC SPERM INJECTION (ICSI).

Introduction: The DAZ gene cluster on the Y chromosome is deleted in 13% of azoospermic men. We, therefore, wished to determine what impact these deletions might have on ICSI patients.

Materials and Methods: 160 infertile men with azoospermia or severe oligospermia underwent Y chromosome mapping. 125 were azoospermic. 51 of these azoospermic men chose to have testicular sperm extraction with ICSI performed, and 28 of 35 men with severe oligospermia (>1 million/cc) underwent ICSI with ejaculated sperm.

Results and Conclusion: Of the 125 azoospermic men (10%), and two of the 28 severely oligospermic men (5.7%) were found to have deletions of the DAZ gene cluster on the Y chromosome. None of the 100 controls had Y deletions, and none of the parents tested had these Y deletions.

Ten of the 51 azoospermic men (19%) who underwent TESE-ICSI were DAZ deleted. Of these ten, five (50%) had sperm retrievable from the testis, and two (20%) became pregnant. Of the 41 azoospermic men who were not DAZ deleted, 22 (54%) had sperm retrievable from the testis, and twelve (29%) became pregnant. The embryo implantation rate was not significantly different for Y-deleted or Y-intact men.

In those azoospermic men who were Y-deleted, deletions limited to the DAZ region were associated with the presence of sufficient testicular sperm for ICSI. Larger Y deletions extending beyond the DAZ region were associated with a total absence of testicular sperm. This suggests that there are several genes on the non-recombining portion of the Y chromosome (NRY) other than DAZ that affect spermatogenesis.

CFTR GENE MUTATIONS IN MEN WITH AZOOSPERMIA OBSTRUCTIVA


INTRODUCTION: One of the causes of azoospermia obstructiva (s.o.) is the congenital bilateral absence of vas deferens (CBVD). This deformation is connected with cystic fibrosis (CF) and its reasons are mutations in the gene of cystic fibrosis transmembrane conductance regulator (CFTR).

Due to literature, 85% of infertile men with a.o. and CBVD have these gene mutations.

MATERIAL AND METHODS: The group of 39 infertile men with azoospermia, from sterile marriages, without any clinical signs of CF, were investigated before the qualification for micromanipulation procedures: ICSI-PESA or ICSI-TESE. In this group, the test of 10 CFTR gene mutations (AF508, G542X, N1303X, 1717-1T, W1282X, G551D, R553X, IS07, R117H and IVS8-5T) using polymerase chain reaction technique (PCR) were done and in 28 patients (from 39) needle testicular biopsies were performed as a stages of diagnostic procedures of azoospermia.

RESULTS: Among 39 investigated patients, in 6 cases (15%) mutation in CFTR gene - AF508 - was detected. Among 28 men after biopsies in 15 cases (53.5%) histopathological findings were positive (i.e. good spermatogenesis) and these men were qualified for ICSI-PESA or ICSI-TESE. In group with positive biopsy, 3 patients (20%) had CFTR gene mutation and their wives were examined as well with negative results.

CONCLUSIONS: The risk of mutations in CFTR gene in men with azoospermia obstructiva due to CBVD, with positive testicular biopsy, is high. So, the examination of CFTR gene mutations is absolutely necessary in these group of patients before the ICSI-PESA or ICSI-TESE procedures.

MICRODELETIONS OF Y CHROMOSOME IN 179 INFERTILE MEN.


Introduction: In 1976, after identification of deletions on the long arm of Y chromosome and Zuffardi postulated the existence of the Azoospermia Factor (AZF). More recently microdeletions of the Y chromosome were found in azoospermic or oligospermic men. We investigate infertile men for microdeletions of Y chromosome in relation with clinical and semen parameters.

Methods: 179 infertile men classified as follows: 52 azoospermic, 54 severe oligospermia, 73 moderate oligospermia or normospermia. The detection of Yq microdeletions was done using a sequence-tagged site (STS) - mapping strategy in a PCR procedure. Moreover, the cytogenetic study was performed on blood lymphocytes on 67 patients. In Yq microdeleted patients a study of SRY (short arm of Y) was also done. Spermograms and clinical investigations including testicular volume were analysed in all patients. 10 fertile men were also analysed.

Results: Using 12 STS we found a microdeleletion of Yq in five secretary azoospermic patients (9.2% in this group) and in 3 severe oligospermic patients (5.5%) with respectively 0.01, 0.02, 0.4 x 106 spermatozoa per ml. The microdeletion were large in 4 cases and only on the Yq chromosome in 4 cases. In 3 azoospermic cases with Yq microdeletion a 46 XY/45X0 mosaicism was found. None of the normospermic men or fertile men have microdeletions. The results of testicular volume, spermogram and cytogenetic studies were reported.

Conclusions: Results from this study show a Yq microdeletions in 5.5% of the azoospermic men and in 5.5% of the severe oligospermic men in agreement with other studies. In time of ICSI method our results emphasise the interest of the genetic studies as the question of transmission of infertility-linked genetic defects may have not only medical consequences but also ethical and psychological.

THE EFFECT OF TAPERED SPERMATOZOA ON THE ACRONOME REACTION, IN VITRO FERTILIZATION RATES, AND EMBRYO CLEAVAGE STAGE IN BOTH STANDARD IVF AND INTRACYTOPLASMIC SPERM INJECTION (ICSI).

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Tapered spermatozoa are observed in the ejaculates of most men, but are often seen with increased frequency in men with varicoceles. Because of the commonness of tapered sperm, our laboratory has been involved in ongoing studies to evaluate the functional capacity of tapered sperm compared to normal, oval-shaped sperm. In this study we have compared the ability of tapered sperm to undergo spontaneous and ionophore-induced acrosome reactions during in vitro culture of sperm from 286 samples from men undergoing IVF. In addition, the effect of tapered sperm on fertilization rates and embryo quality were evaluated and compared for 204 undergoing standard IVF. Eighty-four of the patients underwent intracytoplasmic sperm injection (ICSI). In those patients the morphology of the injected sperm was determined prior to injection, then resulting fertilization rates and embryo quality scores were compared for tapered and normal sperm. The mean percent of spontaneously acrosome reacted sperm following 18 hours of culture was lower as the percentage of tapered sperm increased, however, no significant difference was observed in the level of ionophore-induced reactions. The mean fertilization rate for samples with <30% tapered sperm was 67±4.2 compared to 65.4 ± 5.9 for samples with >30% tapered sperm. The mean embryo score was significantly (p < 0.01) lower for samples with >30% tapered sperm (4.5 ± 0.8) than samples with <30% tapered (5.8 ± 0.4). Interestingly, no difference was seen in either the fertilization rate or resulting embryo quality of oocytes injected with tapered sperm, compared to those injected with normal sperm. These data indicate that tapered sperm may have a decreased acrosome reaction capability, but do not have a decreased fertilization ability with the protocols used for IVF.

The data do indicate an effect on resulting embryo quality.
DECAPITATED SPERM IN RAW SEMEN INDICATES INTRA-CYTOPLASMIC SPERM INJECTION (ICSI) SUCCESS. D.S. Karabinus, C. Racowsky*, L.A. Rudzitis*, and T.J. Geley*, OB/GYN Department, The University of Arizona, Tucson, AZ.

Although sperm morphology reportedly has little effect on ICSI success, sperm neck-region abnormalities are tied to impaired embryo development after ICSI. Since decapitated sperm may be considered the most severe of sperm neck abnormalities the present study was conducted to determine whether the incidence of decapitated sperm in raw ejaculated semen (n=55 specimens; 36 patients) affected ICSI outcome. Inclusion criteria were severe oligozoospermia, severe asthenozoospermia, and/or previous failure to fertilize in conventional IVF. Only the most normal-appearing of available motile sperm were selected for injecting MII oocytes (n=588). Sperm concentration and motility were determined by CASA. Specific sperm morphology traits were recorded for those sperm judged to be abnormal by strict criteria. Raw semen averaged (±SEM) 40.0±5.2x10^6 sperm/mL, 32.6±3.0% motility, and 7.9±1.1% normal forms. Predominant abnormalities were large oval heads (29.8±1.9%), pyriform heads (13.0±1.1%), asymmetrical heads (12.1±1.1%), decapitated (9.7±0.9%), amorphous heads (9.8±0.8%), and tapered heads (7.8±0.9%). Data were stratified by incidence of decapitated sperm in raw semen (≤5%, n=17; 6-10%, n=18; >10%, n=20). Sperm concentration and motility decreased with increased level of decapitated sperm. Fertilization rate was greater for the ≤5% stratum than for other strata (71.9±1.9% vs. 65.5±1.9% and 66.3±1.9%, P<0.05). Pregnancy rate tended to decrease in the higher-strata strata (≤5%: 35.2±1.9%, 6-10%: 27.8±0.9%, and >10%: 25.0±9.9%), but did not differ for patient age, number of oocytes retrieved or injected, embryo cleavage rate, or incidence of the other predominant abnormalities. When the other predominant abnormalities were similarly stratified, there were two significant among-strata differences. Results provide further evidence that abnormalities of the sperm neck adversely affect ICSI success. The incidence of decapitated sperm in the ejaculated population may infer a degree of reduced competence of the intact, normal-appearing sperm in that population to support normal fertilization and the establishment of pregnancy.

HUMAN SPERM-HEMZONA BINDING SELECTION AGAINST SPERMATOZOA WITH CHROMOSOMAL NUMERICAL ABERRATIONS. Q.F. Van Dyk*, C.M. Mahony, S. L8117.endorf", P. Kolm*, D.G. Hodgen•. The Jones Institute for Reproductive Medicine. Department of Obstetrics and Gynecology and Office of Biostatistics, Eastern Virginia Medical School, Norfolk VA.

Spermatozona-Zona pellicula binding selective for human spermatozoa with progressive motility, normal morphology and functional competency. We hypothesized that this gamete interaction would also act to select against spermatozona with chromosomal numerical aberrations. Methods: Ejaculates from 45 patients undergoing ICSI were processed by standard swim-up technique. After processing, the Hamizona Assay was performed with the motile fraction obtained for ICSI. The aneuploidy incidences for chromosomes X, Y and 18 in the swim-up (SU) and pellet (pellet) fractions and sperm bound to the hemizona (HZ-sperm) were compared using fluorescent in situ hybridization with commercially available centromeric probes and DAPI II counterstained (Vysis). Data were analyzed by Chi-square analysis. The relationships between aneuploidy incidence and other semen parameters, fertilization rate, and pregnancy outcome were also evaluated and analyzed using Spearman's rank correlation. Results: Chromosomal numerical aberrations were significantly decreased in HZ-sperm (0.7%) compared to SU (2.2%) and the pellet (2.8%) fractions, P<0.0001. The aneuploidy incidence for the sex chromosomes and chromosome 18 of HZ-sperm (0.61% and 0.10%, respectively) was significantly lower than those of the SU (1.41% and 0.60%, respectively) or pellet (1.69% and 0.72%, respectively) fractions, P<0.0001. The incidence of diploidy of HZ-sperm (0.07%) was significantly lower than that of SU (0.35%) but not SU (0.26%) fraction, P<0.01. Fertilization rate was negatively correlated with incidence of HZ XY (r=0.45, P=0.003) and 46,XY (r=0.41, P=0.007). Pregnancy outcome was negatively correlated with 46,XY (r=-0.38, P=0.013) and diploidy (r=-0.41, P=0.008). Conclusions: There is evidence to indicate that the human spermatozona-Zona pellicula interaction serves as a barrier against aneuploid spermatozoa and indicates the clinical use of Hamizona binding as a filtering device to exclude abnormal sperm.


The black-footed ferret (Mustela nigripes), once considered extinct, has benefited from intensive captive breeding and reintroduction efforts. Despite the overall success of the program, many prime breeding age males (1-3 yr old) do not sire offspring within a given breeding season, resulting in the loss of valuable genetic material. Our objectives were to: 1) determine the % of 1-3 yr old males not siring offspring within a given year regardless of having the chance to breed; 2) determine the % of males (non-siring offspring for 3 consecutive years (1995-1997); 3) compare ejaculate traits in males which have sired offspring (proven breeders); P with males which have not sired (non-proven; NP); and 4) use isaparcous intrauterine artificial insemination (AI) to enhance reproductive In NP males. To obtain breeding histories, individual animal records for 1995-1997 were reviewed. For semen evaluations, electro-ejaculates from 29 P males and 21 NP males were assessed for motile sperm (ejaculate (MSE) and % normal sperm (NS)). For AI, 17 females in natural estrus were treated with 90 IU HCG to induce ovulation and inseminated with semen diluted in an egg-yolk diluent from 9 NP males. A high proportion of males failed to sire offspring in 1995 (54.9%), 1996 (55.1%) and 1997 (59.3%). Further, 21.1% of males born in 1994 failed to sire young in all three breeding seasons. Mean MSE and NS were similar (P>0.05) for P males (6.3±0.5%, 53.5%, respectively) and NP males (8.1±0.1%, 55.8%, respectively). Compromised reproductive efficiency was due to behavioral incompatibility, poor breeding position, poor testes development or copulation with no resulting pregnancy. Thirteen of the 17 (75.5%) females inseminated became pregnant and produced 35 kits (mean litter size, 2.7). Results demonstrate that: 1) captive black-footed ferret males exhibit a high incidence of reproductive failure within and across breeding seasons; 2) efficiency of reproductive failure is not due to semen quality; and 3) AI is effective for enhancing reproduction in NP males. (U.S. Fish & Wildlife Service, Phoenix Zoo, M. & C. Kelley)

DECREASED EXPRESSION OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN THE TESTES OF AGED RATS. A. Lachenko, B.F. Hales and B. Robaire, Department of Pharmacology and Toxicology, McGill University, Montreal, Canada.

While aging is associated with decreased cell proliferation, the reason for age-related oligospermia remains unclear. The objective of the present study is to determine whether this oligospermia is due to the diminished ability of spermatogenesis to undergo mitotic and/or meiotic divisions. Testes of 3 (young), 18 (aging) and 24 (aged) month old Brown Norway rats were examined immunohistochemically for the presence of PCNA, an essential component of DNA replication and repair machinery, at different stages of spermatogenesis. In all rats, intense labelling is detected in the nuclei of spermatogonia and pre-pachytene spermatocytes at all stages. The labelling gradually decreases in pachytene spermatocytes between stages VI-VIII and becomes completely undetectable by stage IX. No labelling is detected in late spermatocytes or spermatids. The incidence of labelling is affected by the aging process in a stage-specific manner. At stages IV-V, the percentage of labelled spermatogonia A1 decreases significantly from 70% in the young to 57% in the aging and 47% in the aged rats. At stages XI-XII (preceding mitosis 2), the percentage of labelled spermatogonia A2 is 23% In the young rats and does not change with age. This suggests that, with age, fewer spermatogonia are capable of undergoing the first mitotic division; however, those that retain this ability undergo the second mitotic division. During S-phase of meiosis, the incidence of labelled preleptotene spermatocytes (stage VIII) is 79% in young rats; decreases to 63% In aging and to 50% in aged rats. Thus, with age, fewer cells are able to replicate DNA when entering meiosis. Further, the pattern of labelling differs in the three groups. In the young rats, labelling is uniform around all tubules; in the aging and aged rats some tubules contain cohorts of unlabelled cells. Thus a decrease in labelling precedes the regression of the tubules. In conclusion, the ability of spermatogonia to replicate and/or repair their DNA before the first mitotic and the first meiotic divisions declines with age. Age-related oligospermia seems to result from a depression of DNA synthesis and repair. Supported by NIH (50423) and by FCAR (Quebec).
**DIFFERENTIAL EXPRESSION OF BCL-2 AND BAX IN THE RAT SEMINIFEROUS EPITHELIUM**

A.P. Sinha Hikim, Y.H. Lue, L. Smith*, J.H. Park*, C. Kung*, G. Nam*, C. Wang, and R.S. Swerdloff, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA.

Programmed germ cell death (apoptosis), in general, is regulated by the Bcl-2 family of proteins which contains both anti-apoptotic (Bcl-2) and pro-apoptotic family members (Bax). In this study, we examined the in vivo expression of Bcl-2 and Bax during normal spermatogenesis of adult rat. Immunostaining was performed on Bouin’s fixed, paraflin embedded testicular sections using affinity purified rabbit polyclonal Bcl-2 or Bax antibody. Negative Controls were run in every assay. In some cases, the primary antibody was incubated with a 10-fold excess of antigen overnight (according to the manufacturer’s instructions), thus providing additional control of immunospecificity. Both these proteins were localized in the cytoplasm in a granular or punctate pattern suggestive of association with intracellular organelles in various germ cells and somatic cells. Among germ cells, Bax immunostaining was most striking in both round and elongated spermatids, whereas little or no Bcl-2 immunoreactivity was seen in these cells. Interestingly, intense Bcl-2 immunostaining was observed in mid (VII-VIII) and late (IX-XII) pachytene spermatocytes, in diploctene (XIII) and secondary spermatocytes (XIV). Unlike Bcl-2, only moderate Bax immunoreactivity was noted in these cells. Also, moderate Bax immunostaining was seen in the spermatogonia as well as in the early primary (preleptotene/leptotene/zygotene) spermatocytes, in contrast to Bcl-2 which was essentially absent in these cells. Sertoli and the Leydig cells contained high levels of both Bcl-2 and Bax. These data show that Bcl-2 and Bax proteins are differentially expressed in the germ cells and suggest that the ratio of these two proteins may be a critical determinant of cell fate during normal spermatogenesis and during spermatogenic regression.

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**HUMAN RNA BINDING MOTIF (RBM) GENE ON Y CHROMOSOME: PROMOTOR DNA SEQUENCE AND FUNCTION IN GERMLINE CELLS.**

WE Taylor*, T. Robarta*, M. Mavita*, I. Hinhazab*, and S. Bhains, Endocrinology Division, Charles R. Drew University of Medicine and Science, Los Angeles, CA 90059

The RNA binding motif (RBM) gene is expressed specifically in germ cells of the mammalian testis, and plays an integral role in male fertility. Multiple copies (25 to 30) of the human RBM gene are located on the long arm of the Y chromosome in interval 8 which is deleted in some infertile men who lack germ cells (sertoli cell only). We do not know how the RBM gene produces its function, nor how gene expression is regulated. To determine the critical region of the promoter needed for gene expression, we cloned and characterized the 5’ upstream regulatory region of an RBM gene. The human RBM promoter sequence on a 1.6 kb DNA fragment was fused upstream from the luciferase reporter gene on vector pXPl. The site of fusion was 50 bases downstream from the putative transcription start site for the RBM gene. A series of deletions of the promoter were fused to luciferase, with lengths of 1.6 kb, 1.5 kb, 1.4 kb, 0.97 kb, 0.7 kb, 0.43 kb, 0.2 kb, and 0.18 kb. We utilized a mouse spermatogonia cell line GC2 to examine the promoter’s overall activity, because this cell line has been shown to express androgen receptors. The series of DNA plasmids were transfected into GC2 cell nuclei using liposomes. A luciferase assay was used to measure the activity of the promoter fragments. Segments from 1.8 to 1.4 and the 0.97 to 0.16 portion contained activator sequences, and the region from 1.4 to 0.97 had repressor activity. Deletion of an Sp1 site on the 0.2 kb region inactivated the basal promoter. Transfection experiments followed by cell growth in media with various ligands indicated that the promoter is not regulated by ligands testosterone and TPA, although forskolin (cAMP) showed some stimulation (80%). We conclude that the promoter region necessary for activating RBM gene expression is the 430 base pair region proximal to the start of transcription, which contains several putative DNA elements for transcription factors Sp1, AP2, and AP1. However, the upstream region may be required to regulate tissue specificity of RBM expression in germ cells. (Supported by grant # GM08140-22 from NIGMS)
CLONING AND PARTIAL CHARACTERIZATION OF A CONSERVED NOVEL HUMAN GENE WITH A UNIQUE TESTICULAR TRANSCRIPT AND HOMOLOGY TO RNA SPlicing AND RNA BINDING PROTEINS

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Using a yeast meiosis complementation assay we isolated several human testicular cDNAs that appear to overcome an RMEI (Regulator of Meiosis 1) inhibition of meiotic recombination. This finding was not consistently reproducible. The size of one of the cloned cDNAs was 743 bp. Using 3' RACE the sequence was extended to a total of 1198 bp containing a 215 amino acid (aa) open reading frame. Using Blast and Benfit sequence analysis algorithms, conservation was demonstrated in the first 80 aa with RNA splicing and RNA binding proteins. The homology in this portion of the protein was 66% identity and 67.5% similarity. Similarity to human spliceosome Associated Protein (SAP-A9) and 36% identity and 57.5% similarity with YAF2 (YHRM2/spennatogenesis factor), and a similar homology with the yeast RNA binding protein PAB1. This portion of the protein contains the RNP (Rhoonucleoprotein) consensus domain. Zoo bloos probed with the 743bp fragment showed hybridization with all species tested except chicken. This indicates conservation among yeast and all of the tested mammalian species.

HYBRIDIZATION of two multiple tissue human northern blots containing 16 different human tissues showed two transcripts (3.7 and 2.1 kb) present in all tested tissues, and a unique 1.1 kb transcript uniquely and abundantly expressed in the testes albeit a very faint expression was observed in polyomavirus-infected cells. 3' RACE is being done to characterize the alternate transcripts and in-situ Hybridization will be done to reveal the cell specific expression of this cDNA.

ROUND SPERMATIDS FROM PREPUBERAL MOUSE TESTIS CAN DEVELOP INTO NORMAL OFFSPRING

I. Sasagawa, Y. Adachi*, T. Tateno*, Y. Kubota* and T. Nakada*, Department of Urology, Yamagata University School of Medicine, Yamaga 99023, Japan.

The male gamete that has just complete the second meiotic division in the testis is the round spermatid and therefore the nucleus of the round spermatid contains a complete haploid set of chromosomes. It was demonstrated that the round spermatids microsurgically injected into mature oocytes participate in syngamy with normal embryonic development thereafter. We investigated fertilization and embryo development after intracytoplasmic injection of rounded spermatids collected from mice before puberty. Spermatids were collected from 16, 18, 20 and 22 days old mice (B6D2F1). Each nucleus was injected into mature oocyte (B6D2F1, 8-12 weeks old) after electro-stimulation. As control, 70-day-old mature males were used. The incidence of normal fertilization and embryo development was about the same when the oocytes were injected with round spermatids from mature or prepuberal males. The rates of development of 2-cell embryos to term were also about the same, regardless of the origin of round spermatids injected. It is suggested that round spermatids from the prepuberal testis already have the ability for normal fertilization and embryo development.

CHARACTERIZATION OF REP38: A RABBIT EPIDIDYMAL PROTEIN PRESENT ON SPERMATOZOA

B. Nitsch*1, H.G. Clarke1, R.C. Jones1* and M.K. Holland2, 1Department of Biological Sciences, University of Newcastle 2Weizmann Biocolstric Cooperative Research Centre, Canberra, Australia.

Exposure of sperm to the epididymal microenvironment is a prerequisite for the development of fertilizing capacity in most mammalian species. A number of proteins have been implicated in this process of post-testicular sperm maturation. In this study we have isolated and partially characterized a rabbit epididymal protein, REP38, which interacts with sperm and which appears to be involved in gamete interaction at the level of the oocyte plasma membrane.

REP38 was identified by SDS-PAGE as a band of M, 38000 present in rabbit epididymal fluid and also present in protein extracts from the plasma membrane of mature sperm. The protein was partially purified by preparative SDS-PAGE and electroelution and used to generate a polyclonal antisem in mice.

Using this antisem and indirect immunofluorescence, intense staining was detected within the supranuclear region of principal cells and on sperm from the distal caput and corpus epididymids. In the cauda epididymal fluorescence was restricted to the luminar border of principal cells. Fluorescence was observed on the head and mid-piece of meatal fixed caudal sperm and this labelling pattern remained unaltered by both ejaculation and capacitation. No staining was observed within the cells of the testis or on testicular sperm.

REP38 antisera had a significant, dose dependent, inhibitory effect on in vitro fertilization (Table 1). Numbers of sperm bound to the zona pellucida (ZP) were not affected by REP38 antisera, nor were the number of sperm in the perivitelline (PV) space. This suggests anti REP38 acts to prevent gamete fusion.

Table 1. Results of IVF trial

<table>
<thead>
<tr>
<th>Animal</th>
<th>Repro. concentration</th>
<th>Repro. eggs</th>
<th>Dist. caput sperm bound</th>
<th>Mean SV sperm bound in ZP</th>
<th>Mean ZP bound sperm in the PV space</th>
<th>% Mean fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>0.02 µg</td>
<td>10</td>
<td>2.9</td>
<td>5.0 ± 2.3</td>
<td>100 ± 10</td>
<td>50</td>
</tr>
<tr>
<td>Addition</td>
<td>0.05 µg</td>
<td>10</td>
<td>3.4 ± 2.3</td>
<td>85 ± 4.0</td>
<td>18</td>
<td>100 + 2.1</td>
</tr>
<tr>
<td>REM30</td>
<td>0.08 µg</td>
<td>11</td>
<td>2.7 ± 2.1</td>
<td>83 ± 2.3</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>REP38</td>
<td>0.08 µg</td>
<td>11</td>
<td>2.7 ± 2.1</td>
<td>83 ± 2.3</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

TELOMERASE ACTIVITY IN THE SEMINAL VESICLE OF INTEGRAL ADULT BROWN NORWAY RAT: REGIONAL DISTRIBUTION AND AGE-DEPENDENT CHANGES

P. Banerjee*, S. Banerjee*, B.R.Zarkin and T.B.Brown, Division of Reproductive Biology, Johns Hopkins University, Baltimore, MD.

Telomerase activity is essential for protection of cells against the telomere erosion that occurs with each round of cell replication, and thus appears to play a role in the indefinite replication potential of some, but not all, eukaryotic cells. In this regard, some tissues contain stem cells that have a long proliferative life-span and are capable of regenerating or renewing the somatic epithelial cell population within the tissue. Because the adult seminal vesicle (SV) exhibits the ability to regenerate during androgen-replacement after castration, we hypothesized that a pool of cells with regenerating potential and which express telomerase activity is present in the adult SV. In this study, using a PCR-based telomerase (TRAP) assay, we show that telomerase activity is, indeed, present in the normal adult rat SV, but only when SV fluid is extruded. Using lung extract as a positive control, we observed a dose-dependent decrease of telomerase activity when increasing amounts of SV fluid protein were added to the assay mixture; 1 µg of SV fluid caused an 85% reduction in telomerase activity. The inhibitory factor(s) present in SV fluid remain to be identified. Telomerase activity in the SV was found to change with age. Compared to activity present in the lung tissue extract (100%), telomerase activity in the SV of young (4 mo) and old (24 mo) rats was 48% and 13%, respectively. Telomerase activity was highest in the distal > intermediate > proximal segment of SV in both young and old rats. These results are similar to the rat prostate, telomerase positive cells are present within the adult rat SV, perhaps accounting for the fact that this organ retains the potential to regenerate throughout life in response to androgen replacement (following castration-induced apoptotic cell death). (Supported by NIH grant AG08321)
Abstract withdrawn.
NMDA RECEPTOR PROTEINS ARE PRESENT IN THE UROGENITAL TRACT AND MAY PARTICIPATE IN THE CONTROL OF ITS SMOOTH MUSCLE TONE

The NMDA receptors (NMDARs) act in neurotransmission through the opening of calcium channels in postsynaptic neurons participating in the central control of micturition and penile erection. Binding of excitatory amino acids to NMDAR triggers several biochemical cascades, including the activation of nitric oxide (NO), which in the penis catalyzes the synthesis of nitric oxide (NO), the mediator of corpora cavernosa relaxation. NO may also act in the control of prostacyclin and bladder tone. The aims of this study are to determine whether NMDAR is present in these organs and elicits a NO-dependent smooth muscle relaxation. The corpora cavernosa, bladder body and prostate were excised from adult male rats and homologous human tissue was also obtained. NMDARs were detected by western blot in membrane and soluble fractions from all specimens using an antibody against subunits 2B and confirmed in bladder and prostate by binding assays with NMDA analog (3H-CPG). Blockade of NMDAR with antagonists inhibited the in situ contraction of tissue strips by 0.2 mM norepinephrine (prostate), 0.3 mM norepinephrine (bladder), or 0.01 mM phenylephrine (corpora cavernosa), or by EFS. The tone inhibitors included agents against the following NMDAR sites: transmitter (D-AP7, pentamidine); polyamine (I-ferropredol, low affinity, (-methylamino, antidiuretic, dextromethorphan, ketamine) and high affinity (dizocilpine). The inhibition was not mediated by NO, and was not counteracted by excitatory amino acids. It is concluded that NMDARs or related receptors are present in the urogenital tract and that they may reinforce its smooth muscle tone by a mechanism different from NMDAR effects in the central nervous system.

PHENOTYPE AND GENOTYPE IN 33 AZOSPERMIC MEN WITH ABNORMAL UROGENITAL TRACT

Congenital absence of the vas deferens (CBVD) is found in 97% of cystic fibrosis (CF) male and in infertile male with CBVD a higher frequency of CFTR mutation is reported to indicate a molecular screening in these patients. The aim of our study is to describe the precise phenotype of these patients in relation to the genotype.

Patients & Methods: Retrospective study of 33 obstructive azoospermic men submitted to CBVD diagnosis. Intracytoplasmatic sperm injection was also performed. Phenotypes were established from the following: history, clinical examination, semen and hormones analysis, seminal biochemical markers, karyotype and/or testicular biopsy, semen and hormonal analysis, and sperm recovery before chemotherapy and at least one specimen during the follow-up period (mean: 653.4 days, range: 170-1418). Sperm characteristics were analysed according previously described methods. Results: No mean sperm values statistically differ between before and after chemotherapy. However considering the individuals values, azoospermic group before chemotherapy: 6 patients are always oligospermic after, while 3 have higher sperm count with in two cases an important improvement. In normospermic group before treatment 4 out of the 7 patients have a reduction on their sperm count which only one presenting a moderate oligospermia 456 days after the chemotherapy. Conclusion: It appears that spermatogenesis is not drastically affected by chemotherapy consisting in two courses of BEP. The sperm values obtained during the follow-up are mainly correlated with the sperm characteristics existing before chemotherapy. Only two patients with severe oligospermia have a high improvement of their sperm count suggesting a testicular tumour effects on spermatogenesis before treatment. Adjuvant chemotherapy with two BEP courses was an interesting alternative in treatment of stage I NSGCT without important spermatogenic toxicity.

TESTICULAR CANCER TREATMENT: EFFECT OF TWO COURSES OF BEP ON SPERMATOGENESIS


Introduction: In patients with high risk stage I non-seminomatous germ cell testicular tumors (NSGCT) adjuvant chemotherapy using two courses of BEP (Cisplatin, Etoposide, bleomycin) appears an interesting treatment to prevent relapse. Although the long term toxicity was good very few data have been reported about sperm recovery. The aim of our study is to analyse the recovery of the spermatogenesis after two BEP courses. Methods: 16 patients treated by two BEP courses for stage I NSGCT were included. They provided one to three sperm samples during sperm banking program before chemotherapy and at least one specimen during the follow-up period (mean: 653.4 days, range: 170-1418). Sperm characteristics were analysed according previously described methods. Results: No mean sperm values statistically differ between before and after chemotherapy. However considering the individual values the oligospermic patients before chemotherapy: 6 patients are always oligospermic after, while 3 have higher sperm count with in two cases an important improvement. In normospermic group before treatment 4 out of the 7 patients have a reduction on their sperm count which only one presenting a moderate oligospermia 456 days after the chemotherapy. Conclusion: It appears that spermatogenesis is not drastically affected by chemotherapy consisting in two courses of BEP. The sperm values obtained during the follow-up are mainly correlated with the sperm characteristics existing before chemotherapy. Only two patients with severe oligospermia have a high improvement of their sperm count suggesting a testicular tumour effects on spermatogenesis before treatment. Adjuvant chemotherapy with two BEP courses was an interesting alternative in treatment of stage I NSGCT without important spermatogenic toxicity.
PREDICTIVE FACTORS OF SUCCESSFUL TESTICULAR SPERM RECOVERY IN NON-OBSTRUCTIVE AZOOSPERMIC PATIENTS
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Testicular spermatozoa from non-obstructive azoospermic patients plus intracytoplasmic sperm injection (ICSI) is a successful treatment in non-obstructive azoospermic patients. The aim of this study was to see whether there are any predictive factors for successful testicular sperm recovery.

The authors reviewed 78 testicular sperm recovery procedures in non-obstructive azoospermic patients, and analysed testicular volume, serum follicular stimulating hormone (FSH) and testicular histopathology.

Spermatozoa was successfully recovered in 39 out of 78 (50%), complete germ cell aplasia (8 out of 36, 22.2%), maturation arrest (4 out of 9, 44.4%) and severe hypospermatogenesis (27 out of 33, 81.8%). Spermatozoa recovery has no correlation with testicular size or serum level of FSH. In patients with high serum FSH > 45 IU/L or extremely small testicular volume (<3 ml), spermatozoa was successfully recovered. The testicular volume and serum FSH were not good predictive factors.

No strong predictors for successful testicular sperm recovery are available but testicular histopathology was most available predictor.

BENCHMARK DOSES FOR LEAD INDUCED SPERMATOXICITY IN RABBITS

The dose-response relationships for elevated blood lead and semen quality have been developed in the rabbit model. The Benchmark Dose (BMD) has been proposed to replace the no-observed-adverse-effect-level (NOAEL) and to overcome the limitations recognized in establishing a Reference Dose (RfD). Benchmark doses that were associated with a 5% and 10% response (BMDL_{5%} and BMDL_{10%}) for the principal endpoints of semen analysis were determined. Eight treatments were used producing target blood lead levels from 0 to 110 µg/dl. A 5 week pre-exposure period was followed by a 15 week exposure testing period.

Semen analysis revealed that increased blood lead levels resulted in adverse changes in sperm count, volume, motility, and morphology. This report provides a basis for determining the blood lead levels that result in a 5% or 10% impairment in the principal variables of semen analysis. For the 5% response, an average (of sperm count, volume, motion, and morphology) BMDL_{5%} is 19.9 µg/dl and for a 10% response an average BMDL_{10%} is 44.3 µg/dl. RfD's were calculated for elevated blood lead effects on semen quality. A 5% adverse response divided by a uncertainty factor of 10 results in a RfD for blood lead of 1.9 µg/dl (19.9/10=1.9); likewise a 10% adverse response would result in a RfD of 4.45 µg/dl (44.3/10=4.45).

FEATURES OF SPERM CHROMATIN FROM MICE TRANSGENIC FOR AVIAN PROTAMINE
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Mice transgenic for avian protamine [TgN (Prml gal) 223Bri] produce sperm all containing avian protamine but with heterogenous amounts between lines and within the same line. These mice are fertile but at a reduced level due to either altered chromatin and/or altered morphology and motility parameters (Biol Reprod 52:20; 581n press).

Since avian protamines do not contain cysteine residues with -SH groups and consequent intra- and intermolecular disulfide bonding as in mouse chromatin, we hypothesized that the transgenic sperm would show a high susceptibility to DNA denaturation in situ, as has been suggested by other authors (J Androl 13:342). Transgenic sperm showed heterogeneity to susceptibility to DNA denaturation, ranging from near normal to moderate levels; thus, -SH status appears not to be highly correlated with susceptibility to DNA denaturation as determined by the acridine orange based sperm chromatin structure assay (SCSA).

Sperm from six mice (2 wild, 4 transgenic) were Feulgen stained and measured by image analysis. The SCSA derived variation of alpha t (αt = red/red+green fluorescence) was correlated (p<0.01) with the variation of sperm image sphericity, eccentricity and length. The mean of αt was correlated (p<0.01) with the variation of chromatin texture (heterogeneity, condenisty and dark and light blobs) as well as the degree of transgenic expression. DNA denaturation susceptibility is thus related to organization of chromatin structure but appears not to be highly dependent on disulfide bonding.


Sperm capacitation occurs in vivo in the female reproductive tract and may occur in vitro. Capacitation is necessary to enable the sperm to undergo the acrosome reaction, which is the fusion of the sperm membranes and subsequent exocytosis of the acrosome. These events allow the sperm to fertilize the oocyte, however, the molecular basis of capacitation is poorly understood. In the bovine, heparin is considered to be necessary to permit capactitation in vitro. The objectives of this study were to determine the effects of heparin on capacitation and on the motility of fresh bovine sperm. Capacitation was determined by the ability of the sperm to acquire chlorotetracycline (CTC) "pattern B," as detected by fluorescence microscopy (BV filter). To study the molecular mechanisms of capacitation, sperm were electrophoretically 3X (200V, 13 Q, 25 µF) with ATPy3P every hour for 5 h (n = 6). Sperm proteins were extracted, subjected to SDS-PAGE (5%-12%) and autoradiography. Over time, heparin induced bovine sperm capacitation according to the appearance of CTC pattern B (P<0.001; n = 8). Autoradiography of the 3P-labeled gels revealed a group of sperm proteins (45-105 kDa) that were phosphorylated during incubation with heparin.

Motility parameters of capacitating sperm were analyzed by computer-assisted motion analysis (n = 4). Motility decreased with or without heparin in a time-dependent fashion (P<0.05). However, the flagellar beat frequency and the amplitude of lateral head displacement significantly increased with heparin in a time-dependent fashion (P<0.001) and may be indicative of hyperactivation. In conclusion, the phosphorylation of a specific population of sperm proteins and motility changes seem to be associated with capacitation of bovine sperm. (Supported by NSERC of Canada. Special thanks to the Centre d'analyse biologique du Québec for semen and technical assistance.)
IMPACT OF PORCINE SEMINAL PLASMA ON THE CONSERVATION OF FRESH SPERM. J.L. Bailey, F. Simard* and G. Arsenault*. Centre de Recherche en Biologie de la Reproduction, Département des sciences animales, Université Laval, Québec, QC G1K 7P4.

In vitro to peptide is enhanced. For poultry, the significant. Hence, brief exposure of washed or Percoll-processed concentrations of SP derived from the pooled whole ejaculates from Quebec, sperm to FertPlus™ peptide increased % sperm bound in the SBA samples gave a substantial increase through Percell did increase quality (80 vs 65% motile), and for 5 of 9 to 640 pM, binding was % sperm bound was increased by each dose of peptide (20-640 pM) for sperm diluted with IO-fold the normal volume of SP.

This study was performed to test the hypothesis that prolonged exposure to SP does not affect sperm quality. Sperm-rich ejaculates (n=7) were diluted with BTS into AI doses containing increasing concentrations of SP derived from the pooled whole ejaculates from 4 different boars. Controls contained BTS + sperm, without additional SP. Treatments were as control but received 2-, 5- and 10-fold the volumes of SP. Diluted semen was stored for 5 days. For each treatment, % motile sperm and viability were evaluated daily. On day 5, the ability of the sperm to undergo the A23187-induced acrosome reaction (AR) was assessed. Motility decreased over the 5 days only in the presence of a 10-fold volume of SP (P=0.0001). On days 2-3 when AI is usually carried out, motility was lower from semen containing a 5-fold volume of SP as compared to control (P=0.05). Motility was poorer from days 2-5 for sperm diluted with 10-fold the normal volume of SP (P=0.0007).

Neither sperm viability nor rates of spontaneous and induced AR on day 5 6 were affected by SP (P=0.05). These results demonstrate that the increased moderate quantities of SP to AI doses has no detrimental effects on sperm quality and conservation of fresh porcine semen. (Supported by CORPAQ.)


Canine epididymal sperm serves as a model for the routine post-mortem sperm collection and IVF in endangered carnivores. To maximize the success rate of IVF the time and temperature requirements for in vitro capacitation must be understood. It has been reported that capa citation of ejaculated dog sperm occurs in 4-7 hr, however the time required for capacitation of epididymal sperm is not known. In addition, temperature regulation of the epididymis may require a co-radiation of IVF procedures. For these experiments, capacitation was confirmed by lophorone induced acrosome reaction. Sperm were stained with Coomasie blue and acrosomes were classified as intact (I) or responding (R, including reacted sperm and those in the process of reacting). In Experiment 1, 4 concentrations of capacitated and ejaculated epididymal sperm were compared. Ejaculated semen was extended in room temperature BWW medium. Epididymal sperm was collected by mincing canine epididymides of castrated dogs into BWW. All samples were evaluated within 2 hours for initial motility, and assessed for vitality using an eosin-nigrosin stain (Nilve). Samples with motility lower than 5% and viability were incubated at 37°C for 0 (To) and 4 hours (T4). In Experiment 2, the effect of temperature on capacitation of epididymal sperm was examined. Sperm was processed as in Experiment 1. For each of three replicates sperm from 1-5 dogs was pooled and equilibrated for 4 hours at 20°C before dilution. In both experiments, at each time interval, an aliquot of sperm was exposed to calcium ionophore A23187 (50 nM) for 5 minutes at 20°C. Simultaneously, untreated incubated sperm served as a control (Con) to assess the rate of spontaneous acrosome reaction. Following incubation, sperm was evaluated for motility, % live, and acrosome status. In Experiment 1, at T5 significantly more (P<0.0001) ejaculated sperm (78.6%) than epididymal sperm (53.6%) responded to ionophore.

Although the difference remained significant (P<0.005) at T7, the response to ionophore by epididymal sperm (72.3%) was approaching that of ejaculated sperm (82.4%). Interestingly, at both times, sperm from the two Con groups exhibited similar rates of spontaneous acrosome reaction. In Experiment 2, low levels of spontaneous acrosome reaction and high levels of ionophore induced reactions were observed at each temperature by T2. Sperm capacitated in BWW at 4°C for 4 hours had increased over T1 in all groups, with the 37°C group increasing significantly more than 4°C groups (P<0.033). At T5, 86-98% of the induced reaction potential had occurred, indicating that capacitation was nearly complete after 4 hours of incubation. The addition of ionophore did not significantly decrease the %live and only slightly decreased motility. These results indicated that the requirements for in vitro capacitation of epididymal and ejaculated sperm were similar and that epididymal sperm can capacitate over a wide temperature range.
FERTILITY IS IMPROVED AFTER SUPPLEMENTING WHEAT SEED DEHYDRATION-INDUCED PROTEINS TO TURKEY SEMEN STORED 24 HOURS IN VITRO

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Dehydration acclimation is a common method used by plants and animals to tolerate changes in environment. Many dehydration-induced proteins are hydrophilic and protect plants during dehydration stress. The object of this study was to determine if proteins from desiccation-tolerant wheat seeds could protect turkey sperm and improve survival and function after liquid storage. Proteins were isolated from wheat embryos (MW cut off 12,000–14,000) and the addition of BSA. Semen was evaluated before and after 24 h of storage for membrane integrity, hypoosmotic membrane integrity, and function after liquid storage. Proteins were isolated from wheat embryos (MW cut off 12,000–14,000) and the addition of BSA. Semen was evaluated before and after 24 h of storage for membrane integrity, hypoosmotic membrane integrity, and function after liquid storage. Proteins were isolated from wheat embryos (MW cut off 12,000–14,000) and the addition of BSA. Semen was evaluated before and after 24 h of storage for membrane integrity, hypoosmotic membrane integrity, and function after liquid storage.

INHIBITION OF ACROSIN ACTIVITY ON HUMAN SPERMATOZOA BY PROTEIN C INHIBITOR

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Introduction and Objective: Protein C inhibitor (PCI) is a heparin binding plasma serine protease inhibitor, which was originally identified as an inhibitor of activated protein C. PCI has a broad protease specificity, inhibiting several proteases in haemostasis and fibrinolysis by acting as a physiological enzyme activator. Recently, it has been described that proteases of the reproductive system such as acrosin, prostate specific antigen and tissue kallikrein can also be effectively inhibited by PCI. However, a direct relation between PCI and the physiological events during fertilization has not been established. An attempt was made to monitor and localize the expression of PCI in the reproductive system and its potential contraceptive spermostatic activity.

Conclusions: Combination of the potenti inhibition of acrosin and the sperm-egg binding by PCI and the localization studies suggested that PCI may protect spermatozoa against premature acrosome reaction and degradation, thereby modulating the acrosome activity so that it can coincide with binding to the oocyte. We hypothesize that PCI los ses its activity after ejaculation, thereby avoiding interference with fertilization.

DOSE RESPONSE EFFECTS OF GRAMICIDIN-D, EDTA AND NONOXYNOL-9 ON SPERM MOTION PARAMETERS AND ACROSOME STATUS

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Previous reports showed that gramicidin-D (G-D), a polypeptide with antiviral and antimicrobial properties, nonoxynol-9 (N9) a common spermicidal detergent, and EDTA, a Ca-Mg chelating agent, inhibited sperm motility and cervical mucus penetration (Bourinbaiar and Lee, Contraception 54: 367-372, 1996). The purpose of this study was to determine the dose response effects of G-D, N9, EDTA and G-D + EDTA on sperm motion parameters and acrosome status. Semen specimens from known fertile donors (n=6) were subjected to CASA analysis (HTM-2030; Hamilton-Thorn Research) of motility (%), path velocity (Vcl, um/sec), progressive velocity (Vcl, um/sec) and hyperviscosity (%). An aliquot of each was incubated at RT for 2 min in G-D (0.04, .01, .04, .4, 4 ug/ml) in PBS, and in Ca-Mg free PBS; EDTA (25, 50, 250, 2500, 3000 ug/ml); G-D + EDTA (0.1ug) in 1% EDTA; and N9 (0, 20, 100, 1000, 2000 ug/ml), then assessed for CASA. Each specimen was also prepared for acrosome status using RITC conjugated pisum sativum agglutinin (PSA) (Benoff et al. Fertil Steril 59:854-862,1993). There was a significant decrease in motility by G-D in Ca-Mg free PBS, EDTA, G-D + EDTA and N9 at all doses as compared to the fresh specimens. G-D + EDTA resulted in a significant decrease in motility as compared to G-D alone. N9 completely immobilized all sperm at each dose, except at 10µg/ml in one specimen. Vcl decreased in a dose response manner, and was significant only with EDTA (500ug/ml) and N9. Vcl decreased in a dose response manner, and was significant for G-D in Ca-Mg free PBS (4ug/ml), EDTA, G-D + EDTA and N9. HA also significantly decreased in all groups. The majority of sperm remained acrosome intact (59.72% RITC+) following exposure to all doses tested. Exposure to N9 resulted in complete breakdown/release of the acrosomal contents (73.83% RITC+), presumably due to its lytic activity. This study confirms previous reports that G-D, EDTA and N9 significantly impairs sperm motility and motion parameters. The effective 50% inhibitory concentration was seen only with N9, whereas G-D, EDTA and G + EDTA generally resulted in incomplete impairment of sperm motion parameters, except in certain specimens where complete immobilization was seen. At the concentrations used, N9 demonstrated potent spermostatic activity. Gramicidin-D and EDTA should be further studied for their potential contraceptive spermostatic activity.

VIABILITY OF THAWED TESTICULAR SPERM MAINTAINED AT ROOM TEMPERATURE FOR VARIABLE INTERVALS BEFORE FREEZING

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Cryopreserved testicular sperm have been used successfully for ICNI, but freezing equipment is not always available to all urologists. Therefore, several clinicians have questioned whether testicular biopsy specimens may be shipped to a sperm bank. In this report, we examined the optimal time for storage at room temperature before freezing. Approximately 250 mgs of testicular biopsy material was obtained from each of 5 men undergoing vasectomy reversal surgery. About 50 mgs of tissue was shredded and placed in a screw top vial with 0.5 ml of Test Yolk Buffer (TYB). One specimen was examined fresh and the others were maintained at room temperature for 1-4 days prior to staged freezing. The fresh and thawed specimens were squashed with a wooden applicator stick and the TYB was centrifuged and resuspended in 0.1 ml. A droplet was stained with SYBR-14 and propidium iodide. The viable sperm were green and the non-viable sperm were red. The total number of sperm were recorded from 20 fields on each specimen. The total viable sperm were 119, 108, 87, 62 and 20 for days 0, 1, 2, 3, and 4 respectively. The total non-viable sperm were 54, 55, 81, 99 and 91 for days 0, 1, 2, 3 and 4 respectively. The total number of viable sperm was 195 for days 1 and 2 compared to 82 for days 3 and 4. The differences between these totals were significant (chi square = 13.4, P=0.001). In 1 case, there were no viable sperm after 4 days of prefreeze at room temperature. These data suggest that testicular tissue may be shipped to a sperm bank in TYB so long as there is no more than 48 hours between acquisition and freezing. Shipment by overnight mail may be possible.
EVALUATION OF SPERM PREPARATION MEDIA FOR OPTIMUM SPERM QUALITY
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In assisted reproduction, spermatozoa must be effectively separated from seminal plasma because separation permits capitation, a prerequisite for successful fertilization. Percoll was recently withdrawn from the US market because of safety concerns. This study evaluated the quality of sperm separated by two different media and compared it to quality after separation on a Percoll gradient. Sperm characteristics from 10 normozoospermic men were compared after sperm separation on three media: a Percoll-type medium (Percoll, Concept Technologies, La Jolla, CA), Isolate (Irvine Scientific, Santa Ana, CA) and SpermFert (Embryotek Laboratories, Inc., Wilmington, MA). Sperm characteristics examined were: sperm count, percentage motility, curvilinear velocity, lateral head displacement, percentage recovery of motile sperm, viability, hypo-osmotic swelling, and penetration in bovine cervical mucus. Sperm morphology was scored using WHO and Kruger's strict criteria. Sperm motility was examined at 0 minutes to 180 minutes after sperm separation on three media to assess which method retained motility for the longest period. Total motile sperm count, motility, velocity, percentage of normal morphological forms, and swim path were statistically analyzed. The results of this study indicate that Isolate is a good alternative to Percoll for sperm preparation media.

EFFECTS OF ENZYMATIC LIQUEFACTION OF SPERM ON SPERM MOTILITY PARAMETERS. JG Alvarez and R. Strock. Dept. of Ob/Gyn, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Sperm motility parameters in semen are routinely determined by microscopic or computer-assisted sperm analyses following semen liquefaction. However, semen may still retain some degree of viscosity after liquefaction that may influence sperm motility parameters. The objective of this study was to investigate the effects of enzymatic liquefaction of semen on sperm motility parameters. A total of 21 semen samples were obtained from 21 males presenting for infertility screening. Samples were allowed to liquefy at room temperature for 2 hours and divided in two 1 mL aliquots. One aliquot was used as control and the other was treated with 2.5 μg of chymotrypsin and incubated for up to 3 hours at room temperature. Sperm motility parameters, including percent motility and curvilinear velocity (VCL), were scored at 5 and 180 minutes using an HTM-IVOS instrument (Hamilton-Thorne Research, Beverly, MA). Aliquots of 100 μL of control and chymotrypsin-treated samples were also centrifuged at 800 g for 16 minutes and incubated for 16 minutes at 37°C. In a separate set of experiments, 100 μL aliquots of seminal plasma of varying viscosity were also added to sperm from 95% Percoll pellets. The means and standard deviations for measurements obtained in control and experimental samples were calculated and compared by t-test analysis. At 5 minutes, mean semen viscosity, percent motility, and VCL in control samples were 22±4%, 36±4%, and 45±5 μm/s, respectively, and in chymotrypsin-treated samples, 26±3%, 40±3%, and 50±6 μm/s, respectively. At 180 minutes these values were 50±4%, 28±4%, and 39±4 μm/s, in control samples and 38±2%, 42±4%, and 71±7 μm/s in chymotrypsin-treated samples. Differences in control and chymotrypsin-treated values were statistically significant (P<0.05). Percent sperm motility and VCL of sperm from 95% Percoll pellets decreased significantly after addition of seminal plasma with viscosities greater than 14 sec (FSL 01). In conclusion, the results of this study indicate that (i) enzymatic liquefaction of semen results in increased percent motility and VCL, (ii) that this effect is maintained for at least 3 hours, and (iii) that this effect is due to a decrease in semen viscosity. Based on the above, enzymatic liquefaction of semen could potentially be used to normalize semen viscosity and to improve the accuracy and turnaround time of semen analysis.

EFFECTS OF CRYOPRESERVATION ON SEMEN QUALITY IN PATIENTS WITH SARCOMA OR CARCINOMA AS COMPARED TO DONORS
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Treatments, such as chemotherapy, for carcinoma or sarcoma in men increase survival but adversely affect reproductive potential. How cryopreservation affects semen quality in these cancer patients as compared to normal donors has yet to be established; thus, we examined the effects of cryopreservation in patients with different types of carcinoma or sarcoma as compared to donors. Semen specimens were obtained by masturbation from patients with carcinoma (n = 13) and normal donors (n = 50) after 48 to 72 h of sexual abstinence. Liquidified specimens were cryopreserved using TEST-yolk buffer. Prethaw and postthaw motion characteristics were obtained by manual grading with a standard procedure using TEST-yolk buffer. Prethaw and postthaw motion characteristics were assessed with a computer-assisted semen analyzer and results verified manually. Patients were similar to donors in age, ejaculate volume, and duration of sexual abstinence. Prethaw total motile sperm count (median and interquartile range) was significantly lower in patients with carcinoma (46.9 X 106; 12.7 to 84.6 X 106) than in normal donors (59.1 X 106; 23 to 90.8 X 106; P = 0.002). Similarly, postthaw percent motility and linearity were significantly lower in patients with carcinoma as compared to donors (P < 0.05). Postthaw, these characteristics did not differ between patients with sarcoma and donors. In conclusion, patients with carcinoma have poorer prethaw semen quality than donors. Cryopreservation similarly affected normal donors. In patients with carcinoma (n = 21) or sarcoma (n = 26) compared to donors (P < 0.05). Similarly, postthaw percent motility and linearity were significantly lower in patients with carcinoma as compared to donors (P < 0.05). Postthaw, these characteristics did not differ between patients with sarcoma and donors. In conclusion, patients with carcinoma have poorer prethaw semen quality than donors. Cryopreservation similarly affects sperm quality in patients with carcinoma or sarcoma compared to normal donors. Overall, postthaw semen quality is better in patients with sarcoma than carcinoma. Sperm should be cryopreserved before treatment for sarcoma or carcinoma. Patients with carcinoma can possibly achieve pregnancy with simpler assisted reproductive technique (e.g., intrauterine insemination) because they have better postthaw semen quality.

ORIGIN AND DEVELOPMENTAL CHANGES OF HYPO-OSMOTIC SWELLING AND IMPACT OF COMMON LABORATORY TREATMENTS ON SUCH SWELLINGS OF HUMAN SPERM. A.M. Hooman, B. Krik, C. Hauf, and I.H. Thompson*. Dept of OB/GYN, University of South Alabama, Mobile, AL.

Like other sperm function tests, the hypo-osmotic swelling test (HOS test) in its present form does not provide useful information regarding the fertilizing ability of the sperm. This test usually takes into consideration the total HOS response value with no emphasis on the value of the response sub-types. Secondly, the test ignores the possible impact of laboratory handling in scoring the test results. In reality, the ejaculate undergoes multiple laboratory treatments for recovering the sperm from semen. This study investigated the origin and development of different HOS responses and the impact of common laboratory treatments on them. The morphological changes in the sperm tail were monitored by incubating the sperm in the hypo-osmotic solution for 16 different time periods. Also, the semen samples underwent 5 laboratory treatments prior to the standard HOS test. The HOS reactive spermatozoa and the type of HOS reaction (swelling sub-types) of the samples subjected to different experimental conditions were identified under a phase contrast microscope. The HOS response pattern of the aliquots that underwent heat shock, freeze/thaw and Percoll wash were significantly (P < 0.05) different from that of the corresponding control. The prolonged post ejaculation time did not diminish the HOS potential of the spermatozoa. The response subtypes a and g were more affected compared to other responses (b, c, d, e, and f) by the treatments tested. None of the treatments could influence the d and e types of swellings. The 100% HOS response in the first minute of incubation was type b response. During the first 5 minutes, the HOS responses were confined to a, b and c types. From 10 minutes onward, the type d, e, f and g responses were additionally observed. More than 50% of the reacting spermatozoa showed swellings within 25 minutes and incubation beyond that time did not cause significant variation in the relative abundance of the swelling sub-types. These observations provide evidence of time course morphological changes in human sperm tails. The ultrastructure based analysis of the HOS responses indicate that the response sub-types are the apparent reflection of the differences in the cytoskeletal assembly of the sperm tail. The evaluation of the HOS response sub-types may, therefore, help in detecting those physiological variations within a sperm population of a semen sample.
COMPARISON OF THREE TECHNIQUES FOR SPERM CAPACITATION

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The Percoll discontinuous density gradient centrifugation technique has been used as one of the most useful methods in connection with assisted reproduction techniques (ART). However, the manufacturer, Pharmacia, has withdrawn Percoll media for the use in human ART. Consequently, alternative products have been developed.

The objective of this study is to evaluate different sperm capacitation techniques, Swim-up, PureSperm (Sandwichian, Sweden), Ixaprep (Mediput, Denmark), and to compare the results obtained in ART (IUI, In vitro fertilization (IVF), Intracytoplasmatic Sperm Injection (ICSI)) with these three capacitation media.

Sixty nine insemination cycles, fourteen IVF cycles and fifty-eight ICSI cycles were analyzed (motive sperm recovery, fertilization rate, pregnancy rate, implantation rate, etc) in order to compare the effect of the capacitation techniques in the outcomes of human ART.

No differences were observed in the average recovery of motile sperm between Swim-up, PureSperm and Ixaprep, such as no differences were detected in the fertilization rate in IVF and ICSI. The pregnancy rate was similar in all treatments (intrauterine insemination, IVF and ICSI) with the three capacitation techniques.

In conclusions, the new capacitation media, Ixaprep and PureSperm, do not produce better results than the Swim-up technique in connection with assisted reproduction techniques (ART).


Ejaculates from eight dairy bulls were split in half to evaluate the effects of glycerol addition (G+) on sperm organelle function, versus the effects in extended semen lacking glycerol (G-). Although glycerol is the protective agent that helps stabilize sperm membranes during cryopreservation, preliminary data suggested that glycerol had detrimental effects on sperm compartments to varying degrees. To assess compartmental effects, three organelle-specific fluorophores were used to analyze G+ and G- sperm stored 24 hr at 5°C. The mitochondrial probe, 5,5',6,6'-tetrachloro-1',3',5'-tetratriethyl benzimidazolylcarbocyanine iodide (JC-1) provided a more rigorous estimate of metabolic function than rhodamine because it discriminates between relatively high and low membrane potentials. In this experiment, sperm mitochondria were stained with JC-1 and displayed red-orange aggregates or green monomers, identifying high or low membrane potentials, respectively. Intact plasma membranes were identified by SYBR-14-propidium iodide staining. The fluorescence-labeled lectin from arachis hypogaea, PNA-FITC, identified sperm that were acrosome-reacted. Split-plot analysis of variance revealed that the addition of glycerol modified the proportions of sperm that stained with organelle-specific fluorophores. In 24-hr stored samples, the populations of JC-1 aggregates decreased with glycerol addition (p< 0.001) while the proportions of JC-1 monomers increased (p = 0.02). Therefore, total JC-1-labeled mitochondria were similar in G+ and G- samples (p = 0.80). The proportions of SYBR-14-stained sperm were also similar (p = 0.11). The proportions of acrosome-reacted sperm, however, were greater in the G- samples than in the G+ samples as indicated by PNA-FITC (p = 0.03). These findings suggest that the plasmaemmembranes, the acrosome, and the mitochondria of unfrozen sperm varied as to their functional status in response to the addition of glycerol.

DETERMINATION OF NORMAL SPERM MORPHOLOGY (NSM) VALUE BASED ON 25 PRE-VASECTOMY ("FERTILE") Ejaculates

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NSM, though associated with the fertility potential of spermatozoa, is a very subjective assessment. The WHO Laboratory Manual for the Examination of Human Sperm and Semen Cervical Mucus Interaction, 3rd edition, suggests an "empirical value" of 37% or more for NSM as an index for couples' semen analysis. The objective of our study therefore was to establish a NSM value for a local population of fertile men. Subjects consisted of 25 men with proven fertility recruited from a private Urology practice requesting vasectomy. Demographic information, reproductive histories and informed consent were obtained from each individual. One ejaculate was produced by masturbation and evaluated for semen volume, sperm density, total and progressive motility, and sperm morphology. Multiple cytological slides were made simultaneously from each ejaculate, and stained using the Papanicolaou technique modified for semen. Four slides per subject were analyzed by a single technician (CJB). The morphology data obtained were analyzed for repeatability.

Subjects had an average of two children. The average length of time from their youngest child's birth to submission of the study semen sample was five years (range: 1-11 years). Across subjects, the mean ±SD. for sperm concentration was 103 ±65.9 million per ml and for overall motility was 59 ±12.5%. The mean percentage of NSM was 30 ±9.8%. For 17 of the 25 ejaculates studied, the within subject repeatability of the morphology assessment was within 1 S.D. of the mean. Although the mean values obtained for sperm concentration and motility for this study ("fertile") population are above the normal values, NSM will now be reported as ≥50%, based on the repeatability of the morphology assessment (1 S.D. below the mean). "Equivalent" will be defined as 80-20% normal forms, representing those values that fall between one and two standard deviations below the mean. "Abnormal" will be less than or equal to 10% normal forms, representing greater than two standard deviations below the mean of this study population. The cut off values for NSM reflect the statistical meaning of normality, though may not accurately distinguish clinically fertile from subfertile men.

Sperm morphology is an important component of bull semen evaluation. An increase in abnormal sperm is associated with reduced fertility. Manual methods of analysis are subjective and highly variable within and between technicians. To reduce the subjectivity and variability of sperm morphology assessment, computer automated sperm head morphology analysis (ASMA) has been developed. The objective of this study was to determine the Inter- and Intra-analysis and technician variation associated with ASMA of bull sperm heads. Semen from ten bulls was diluted to 200x10^6 sperm/ml, slides smeared and stained using hematoxylin and rose bengal (MGZN; Gravance et al., 1996 Theriogen 46:1205). Each of 2 technicians analyzed 200 sperm from each slide, 3 times, using ASMA. The morphometric dimensions of area (A), perimeter (P), length (L), width (W) and W/L for individual sperm of each analysis were assessed by GLM-ANOVA for effects of bulls, replicates, technicians and interactions. The coefficients of variation (CV) were recorded for each analysis and determined across replications. The mean CVs within and between analysis were compared among technicians by GLM-ANOVA. No differences (P>0.05) between technicians were found between or among bulls for A [20.83μm^2 vs 29.23μm^2], P [3.73μm vs 23.86μm], L [8.75μm vs 8.71μm], W [4.47μm vs 4.65μm], or W/L [0.51 vs 0.51]. No differences (P>0.10) between replicates on sperm head dimensions were detected within or among bulls for either technician. No intra- or inter-analysis differences (P>0.10) between technicians or CVs were observed. The mean intra-analysis CVs for all bulls for both technicians were A=9.9%, P=4.9%, L=4.5%, W=5.6% and W/L=4.5%. The mean inter-analysis CVs for both technicians were A=3.0%, P=2.4%, L=2.0%, W=2.0%, and W/L=1.7%. The results indicate that ASMA is a repeatable and objective method of assessing bull sperm head morphology within and between technicians. No differences between replications were detected, hence replicate analyses are not necessary to acquire accurate morphometric data.

RESULTS

SPERM MORPHOLOGY ANALYSIS - PROBLEMS AS DEMONSTRATED BY PROFICIENCY TESTING D. B. Knizer, C. Caruso, J. Quigley*, S. A. Rothmann, Fertility Solutions Inc, Cleveland, Ohio.

Objective: To analyze proficiency testing (PT) results for sperm morphology and to compare them to reference lab values. Design: Retrospective analysis of results of 5 PT events sent to 725 participants between July of 1995 and July of 1997.

Materials and Methods: Labs were sent 2 concentrations of stabilized sperm (1 low, 1 high) for sperm concentration analysis, and 2 eosin-nigrosin stained slides for sperm viability analysis. They were instructed to return their results along with the methods used for analysis.

Results: The majority of laboratories participating in the proficiency tests were general hospital pathology laboratories that perform body fluid analysis. Sperm concentration results were stratified according to counting chamber used and also by method (manual or CASA). Most observers used a hemacytometer chamber (64%), with the remainder using the Malter (21%), Humagin (8%), Micro-Cell (5%) and CellVu (2%) chambers. 95% of the participants performed manual counts and 5% used CASA. For low sperm concentrations (<15 million/ml), there were slight differences in means from different chambers that increased with higher concentrations. The precision for the low and high concentrations as reflected by standard deviation (SD) was variable for all chambers used. In contrast to published single lab studies of chamber precision, no chamber was consistently more precise among this large group of observers. The accuracy and precision for manual vs CASA results were similar for both high and low concentrations. When reference lab values were compared to the PT values, for high and low concentrations, the means usually were not different but the precision was better in the reference values as expected. Means of results for % viable for all PT events were the same as reference lab values, although variability was consistently about two times higher in the PT group. Discussion: High variability exists in the determination of sperm concentration and viability among labs that participated in our PT. In spite of the large variation in precision generated by the results, 95% of concentration and 3% of viability observations consistently exceeded acceptable limits. These results support the need for better quality control and standards for semen analysis.
Standardization of the work-up of andrology patients and clinical research are indispensable tools for the improvement of the quality of patient care. Clinical research requires the collection of reliable, complete, and unambiguous data. Provided that an infrastructure is present that supports and stimulates collection of structured and complete data.

We have started a standardized electronic data collection in 1988. Analysis of the data was, however, hampered by the scattering of data over several sources. By the development of ARIS (Andrology Research Information System), data was integrated for clinical research.

The frequency distributions of semen parameters, hormone assays, diagnoses, and items of history taking, physical examination, scrotal ultrasonography and varicoceles treatment, were studied for a group of 1549 andrology patients. The data structure in ARIS facilitated the assignment of diagnoses according to WHO diagnostic classification rules. We found positive correlations between testicular volume, FSH, and sperm counts (p<0.01).

Inhibin B serum concentrations showed a stronger correlation with testicular volume, Johnson score, and sperm parameters, than serum FSH. The results imply that Inhibin B is a sensitive endocrine marker for spermatogenesis. Doppler ultrasonography detected subclinical varicoceles, which were treated just as effective as clinical varicoceles in terms of post-surgery improvement of sperm count and sperm motility. Moreover, ultrasonographic scrotal pathologies, Testicular tumors, not detected by palpation, were found in 0.5% of the patients.

The routine entry of structured patient data into electronic repositories results in a valuable data source for clinical research, and a patient approach. The evaluation of the data gives physicians important insight in their population, and is useful for planning, quality assessment and patient care.

A cross-sectional study was conducted to investigate the prevalence of sperm aneuploidy among pesticide factory workers in Anhui, China. We recruited 60 men, 30 from a large pesticide manufacturing plant and 30 from a nearby textile factory. Each subject met the following criteria: between ages 20-40 years, working in the plant continuously for 3 months prior to the study, no congenital anomalies of the external genitalia and no history of recent febrile illness or mumps. Semen evaluation and fresh semen smear slides were made within one hour after its collection from each subject. Slides were subsequently sent to Case Western Reserve University for aneuploidy evaluation. Exposure assessment revealed that the workers in pesticide plant were exposed to ethyl parathion or methamidophos, 2 potent organophosphate pesticides, at the mean level of 0.02 mg/ml (8-hour time weighted average (TWA) using personal pump) and mean end-of-shift urinary p-nitrophenol of 0.13 mg while the workers in control plant had no such occupational exposure. After examining quality of slides, 29 slides were randomly chosen for aneuploidy scoring using 3-color fluorescence in situ hybridization (FISH), employing probes to centromeric alpha satellite repeats of chromosome 18 and X and Y chromosomes. Scorsers were blinded to the exposure status of the workers. The (3 exposed and 6 non-exposed) men had mean semen parameters as followed: abstinence period 5 days (7 days), 145.4±10² (186.8±10²) sperm count, 49% (58%) percentage of normal motility and 59% (60%) percentage of normal morphology. The abnormalities of interest consisted of aneuploidy for chromosome 18, and the three different types of sex chromosome disomy, i.e. XX, XY, and YY sperms. The crude rate of all aneuploidy combined was 0.30% and 0.19% for the exposed and the non-exposed, respectively. Poisson regression with overdispersion adjustment yielded significantly different rates of aneuploidy: 3.03 per 1000 sperms (exposed) and 1.94 per 1000 sperms (non-exposed) giving a rate ratio of 1.56 (95% confidence limit 1.06 - 2.31). The regression coefficients remained unchanged after adjusting for age. Therefore, we conclude that occupational exposure to organophosphate pesticide moderately increases the prevalence of sperm aneuploidy in Chinese workers.

Sperm Aneuploidy Among Chinese Pesticide Factory Workers. Chentara Penagalota1, Terry J Hassold2, Hang Qiu Xu1, Xiping Xu1. 1Department of Obstetrics, Wenhua Hospital, Beijing, China. 2Department of Obstetrics, Case Western Reserve University, Cleveland, OH 44106, USA. Aneuploidy Public Health Bureau, Anhui, China.

**ACBECIAL SPERMATOZOA: ABNORMAL DEVELOPMENT OF THE HEAD-NECK ATTACHMENT WITH FOLLICLE FAMILIAL INCIDENCE.** H. H. Chenes, Laboratory of Testicular Pathology, Burnside Medical Centre, R.C. Hospital, Argentina. Microcephalic" spermatozoa have been occasionally reported as the main seminal abnormality in sterile men. We present a series of 9 patients with this rare anomaly. All of them were young adult males with primary sterility. Morbidity ranged from normal to severe aethanosoospermia. 75-100% spermatozoa from all 9 patients showed very minute cephalic ends ("pin heads") and 0-24% abnomal head-middle-piece attachments. Loose heads amounted to 0-10 each 100 spermatozoa. Normal forms were exceedingly rare. Two patients ware brothers (not twins). Sperm characteristics remained stable for long periods in numerous spermograms. On ultrastructural examination the head was absent from the cephalic end. The anterior aspect of the middle piece ended in a neck region directly covered by the plasma membrane. When present, heads implanted at abnormal angles on the sides or the anterior end of the middle piece. The few loose heads in semen did not show the implantation fossa that normally accommodates the flagellum. In one patient a testicular biopsy was processed for electron microscopy. Spermatogenesis evidenced normal spermatogonia and spermatocytes, but severe alterations in spermatida. Nuclear condensation and acrosome formation advanced normally, but the implantation fossa was never formed. The flagellar anlage developed independently from the nucleus and never migrated to the caudal pole, resulting in an abnormal head-middle-piece connection. Sertoli cell phagocytoeis was intense. In one patient with 1 normal spermatozoo, acrospermia was induced with parenteral testosterone to attempt an increase of the normal sperm clone during the rebound phenomenon, but all newly formed spermatozoa were acphalic. The findings indicate that acphalic spermatozoa of F.M. and (2) whether abnomal head-middle-piece implantations are of testicular origin and arise as the result of an abnormal neck development during testicular spermatogenesis. The familial incidence and the typical phenotype found in all spermatozoa from different patients strongly suggest a genetic origin of the syndrome.
HISTORY OF CRYPTOCHIDISM, SCROTAL TEMPERATURE, SPERMATOGENESIS AND FERTILITY.


The effects of a history of cryptorchidism on spermatogenesis and fertility repeatedly produce divergent opinions. The aim of this retrospective study was to bring clinical and biological information.

Material & Methods: Testis position was recorded in 85 fertile and in 95 men with a history of cryptorchidism among 1014 consecutive infertile men. Testis position was recorded as low when in the bottom and high when in the upper part of the scrotum. The patient was asked whether each testis was spontaneously and regularly ascending up in a supracrotal location, with the physician indicating this area with his finger; a positive answer was recorded as an "inconstant ascending testis". Testes volumes, scrotal temperatures, serum testosterone, FSH and LH were measured. WHO guidelines were used for semen analyses.

Results: The frequency of a history of cryptorchidism was significantly higher in infertile (9.4%) than in fertile (2.4%) men (p<0.01). The volume of a former cryptorchid testis was significantly smaller than the contralaterally normally descended testis. The mean sperm count, motility and normal form were significantly lower in cryptorchid than in fertile men, but mean FSH levels were higher (10.6±6.8 vs 8.1±3.5 IU/L, p<0.001). The frequency of at least one testis in a high scrotal location did not differ between fertile (16.5%) and cryptorchid men (27.2%).

In an inconstant ascending testis was significantly more frequent in cryptorchid (30.4%) than in fertile men (11.8%). In cryptorchid men, an inconstant ascending testis was significantly more frequent on the cryptorchid side, and after hormonal than after surgical treatment. Among the cryptorchid men, 45% had an abnormally high scrotal temperature which was associated with significantly lower total sperm count (79.5±107 vs 28.7±64×10^6/ml, p<0.001) and higher rate of primary infertility (12/51 vs 24/1; OR = 6.00; 95% Cl = 1.19-57.73).

Discussion: In former cryptorchid men, inconstant ascending testis and abnormally elevated scrotal temperature appear to be risk factors for both spermatogenesis and fertility.

VIRAL VECTORED IMMUNOCONTRACEPTION FOR RABBITS

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Fertility control using genetically engineered, species-specific, infectious viruses containing species-specific antigens is an option for controlling animal numbers in species which are geographically widely dispersed. The zona pellucida antigens ZPB is essential to fertilization in the rabbit and thus provides a target for immunocontraception. We produced large quantities of recombinant ZPB which possesses many of the biochemical characteristics of the native molecule, using the vaccinia T7 expression system. This was used to immunize (ZPB 100µg in Freund's Complete Adjuvant followed 30 and 45 days later with boosts in Incomplete Adjuvant) both male and female New Zealand white rabbits. Male rabbits, in which ZPB is a foreign antigen, responded with a rapid rise in serum IgG after the primary injection. These titres increased by several logs with subsequent boosting. In contrast female rabbits did not respond with detectable serum IgG until after the initial boost indicating a degree of immunological tolerance. Final levels achieved in males and females were comparable. At day 60, 75% of treated females were infertile. Interestingly, when the gene encoding rabbit ZPB was inserted into an intracisternal site in the myxoma virus and the recombinant virus used to infect male and female rabbits both sexes responded with a similar rapid rise in serum IgG levels which reached maximum approximately 15 days post infection and thereafter declined clearly the antigen present in the virus overcomes tolerance. At 30 days post infection 25% of these females were fertile. None of the females infected with the parental strain were infertile. However, if animals were boosted by subcutaneous administration of recombinant ZPB protein (100µg) in Freund's incomplete adjuvant at days 30 and 45 post viral infection the antibody response increased several logs in both sexes and at day 60 80% of the females were infertile.

INTACTROPLASMSIC SPERM INJECTION (ICSI) IS AN EFFECTIVE TECHNIQUE TO ACHIEVE PREGNANCIES DESPITE SUBNORMAL HYPO-OZOOSPERMIA.


INTRODUCTION: Urnsplasma urealyticum has been recognized as the etiology of many infections in humans with increasing in turn relate to infertility found on genitourinary tract is M hominis and U. urealyticum. These species can be distinguished from each other by differences on colonial morphology, metabolic features and antibiotic susceptibility. Mycoplasma like structures have been reported on spermatozoa spermatozoa infected by U. urealyticum infection, yet there is not enough evidence to ascertain the exact relationship between mycoplasma infection and human spermatozoon. Pneumocystis of this genome and spermatospermatozoa uteritis have hampered structural descriptions of abnormalities on spermatozoa. The goal of this work is to find mycoplasma like particles on semen samples from infertile males and describe its ultrastructural features.

METHODS: 22 mycoplasma infected volunteers were selected from 74 infertile patients. A sample of semen was obtained by masturbation, and processed by Electron Microscopy (EM) As a control a u. urealyticum positive culture broth was processed for similar morphology studies.

RESULTS: EM observation of centrifugated sediment of seminal fluid revealed 150-280 nm diameter particles spherical to elliptical in shape regular edges and membrane de limited. Their contents are homogenous, moderately electron-dense and found both free in the seminal fluid as well as adhered to cells on the ejaculate. A fixed to spermatozoa tails, mainly to middle piece. Particles were also observed within spermatozoa cytoplasm. Abundant necrobiots were observed often which carried mycoplasma-like particles within phagocytic vacuoles. Mycoplasma morphology was confirmed on ultrastructural analysis of centrifuge pellets from u. urealyticum grown in specific broth.

DISCUSSION: Identification and description of Mycoplasma sp. on biological fluids such a semen is frequently hampered due to heavy autolysis, induced by this microorganism which weak the presence of Mycoplasma. On the other hand, pleomorphism found by other authors on this bacteria may cause difficulties to make a diagnosis on ultrastructure studies of the ejaculate. Our data show that morphological pleomorphism affects the size rather than the shape on appearance of mycoplasma. Thus we suggest that search for urnsplasma urealyticum by ultrastructural techniques be considered as a feasible and important tool for diagnosis.
DELIVERY OF A HEALTHY MALE AFTER OOCPLASMIC INJECTIONS OF SECONDARY SPERMATOCYTES (SSs), N. Sofikitis, 'T. Mantzavinos, ' D. Loutradis, ' I. Stygias, ' Dept. of Urology, Tottori University School of Medicine, Yonago, Japan; ' Dept. of Obst/Gyn, Athens University School of Medicine, Athens, Greece

We applied oocplasmic injections of SSs for the treatment of patients without spermatoozoa and spermatids in their testicular biopsy. A minor oocplasmic stimulation was performed to inject the SS into the oocyte. One hour later, the second penetration of the oocyte followed by vigorous oocplasmic stimulation was applied to activate the oocyte. Twelve oocytes were fertilized, and all of them exposed a male (peudos) polar body. Fluorescent in situ hybridization (FISH) technique showed that the male polar body had haploid (Y) n DNA content. Furthermore, FISH techniques showed that the cells we considered as SSs were chromosomally haploid but had 2n DNA content. Twelve embryos were transferred to the wives of three patients. One pregnancy was achieved. A healthy male was delivered on July 9, 1997. Injections of SSs may serve as an experimental treatment for men with neither spermatoozoa nor spermatids in their testicular biopsy specimens. The male second meiotic division (MID) can be completed within the ooplasm. In this case, one haploid nDNA product of the MID (i.e., male polar body) is extruded into the perivitelline space.

CATALASE ENHANCES BOVINE EMBRYO PRODUCTION
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To further investigate a putative embryotrophic effect after catalase treatment of mouse sperm (Kuribayashi & Gagnon, Fertil. Steril. 66:1012-1017, 1996) catalase was added to fertilization medium and bovine embryonic development was assessed. Immature oocytes recovered from 2-6 mm diameter ovarian follicles at slaughter were selected (127 to 132 per group) for in vitro maturation (IVM), fertilization (IVF) and culture (IVC), or IVMFC, and washed in Medium 199 (M-3769, Sigma Chemical Co., St. Louis, MO) with bicarbonate, pyruvate, gentamicin and PVA. IVM was in the same medium without PVA but with 25 ng FSH (NIDDK-oFSH-17) per ml and r-hIFG-I (100 ng/ml; Promega Corp., Madison, WI). IVF and IVC were similar to Keskinthene & Brackett, Biol. Reprod. 55:333-339, 1996, but sperm capacitation was with 20 µg heparin per ml and 10⁵ sperm/ml were used for a 5 h IVF insemination interval. For IVC, 6 to 12 presumptive zygotes were cultured (3 al per ovum) in IVC medium modified by addition of glutamate (0.5 mM), no glucose and citrate, for the first 67 h. Pen-strep replaced gentamicin throughout. At 72 h and 144 h post-insemination (pi) embryos were transferred to fresh e-COF+NEA. Results (% of oocytes) reaching 4-cells by 72 h, morulae by 144 h, blastocysts by 270 h post-insemination (pi) were obtained for catalase concentrations of 10, 20, and 40 µg/ml. Analyses were by ANOVA and Bonferroni t-test after arcsine transformation. Catalase (80 µg/ml) addition for IVF enabled significant higher (p<0.05) proportion of development at each stage. Results suggest that the use of catalase may lead to compromised bovine IVMFC results in absence of catalase. (Supported by FAPESP, Sao Paulo, Brazil, Genex Cooperative, Inc., Ithaca, NY, Shapiro Packing Co., Augusta, GA, and NHP, NIDDK, NICHID, and USDA).

COMPARISON OF ISOLATE, PERCOLL AND GLASS WOOL SPERM SEPARATION METHODS ON INTRARETINE INSEMINATION OUTCOME.

Objective: The removal of Percoll from the commercial market has created the need for a comparable method of sperm separation for use in assisted reproductive technologies. ISolate (Irvin Scientific) is a colloidal suspension of silica particles stabilized with covalently bound hydrophilic silane. ISolate has recently been approved by the FDA for human use. The purpose of this study was to compare the pregnancy rates (PR) in IUI cycles with sperm processed by ISolate(P), Percoll(P) or Glass Wool(GW).

Design: A retrospective analysis of 292 IUI cycles performed over a 6 month period.

Materials and Methods: Fresh ejaculates obtained from patients reporting to our clinic for intrauterine insemination were analyzed according to WHO guidelines for sperm count, motility, forward progression (FP) and total motile count. The ejaculates were then processed by one of the three methods: ISolate (n=19), Percoll (n=99) or Glass Wool (n=54) separation, according to established protocols. Postwash sperm quality was assessed. Ovarian stimulation included clomiphene citrate (CC), CC + hMG, hMG alone or natural cycle. All patients had patent fallopian tubes. Insemination occurred 24 - 36 hrs. post LH surge or hCG injection.

Results: No significant differences in PR were seen among the three methods of sperm separation. In all cases, post cycle PR were: (ISolate), 10.5% (CC) and 13.2% (CC + hMG). In normal non-spermic samples only, ISolate exhibited a greater PR (13%) than did the other methods (P < 0.01; OW: 9%), although this was not statistically significant. In male factor cases, no significant differences were observed between ISolate vs. OW (9.3%) or P (9.5%) vs. OW (9.6%). However, the PR for male factor sperm processed by P separation (10.3%) was significantly different (p<0.05) from that of OW (6%). No significant differences were observed among the three methods of separation for the recovery of total motile sperm (mean ± S.D.: 46.1% ± 19.5%) or postwash motility (11.4% ± 13.3; P: 9.1% ± 11.9; and OW ± 9.7% ± 11.5). However, the postwash FP was significantly different (p<0.01) for sperm separated by the ISolate method (mean ± S.D.: 93.1% ± 10.9) compared to that of OW (96.2% ± 3.2) or GW (96.8% ± 2.0).

Conclusion: ISolate is as effective as other methods of sperm processing in achieving pregnancy in intrauterine insemination cycles for both non-spermic and male factor cases.


Previous data have demonstrated an increased spontaneous abortion (SAB) rate following conventional insemination of oocytes during in vitro fertilization (IVF) in the presence of oligoasthenospermia. Since some preliminary data suggested that the use of intracytoplasmic sperm injection (ICSI) for subnormal specimens was not associated with pregnancy loss, the etiology of SAB may not be genotype but rather other factors or events related to the zona pellucida. Implantation defects possibly related to these factors may serve as a cause for the increased SAB. However, these data fail to corroborate conclusions by van der Linden (1996). The present study was conducted, patients using donor sperm or exhibiting antisperm antibodies were excluded. A total of 52 pregnant patients who conceived following IUI were included in the study. The SAB rate in the normal group (n=34) was 28.2% and in the low motility group (n=18) was 27.8%. Thus, these data fail to corroborate conclusions by IVF pregnancies that oligoasthenospermia may be a cause of SAB. However, the two conclusions need not necessarily be interpreted as contradictory since there is a large difference in the numbers of sperm reaching the zona pellucida following conventional oocyte insemination with IVF than with IUI. An alternative hypothesis is that the implantation defect does not become manifested until a certain number of defective sperm attach to the zona pellicida.
THE BENEFIT OF VARICOCELE REPAIR ON PREGNANCY RATES IN COUPLES WITH FEMALE PARTNER AGE GREATER THAN 35
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Currently varicocele induced oligozoospermia is frequently treated IVF or ICSI. Many of these couples, are faced with an advanced maternal age (>35). The pregnancy rates with IVF/ICSI are, optimally 50%, and decrease significantly with maternal age. The female partner age and subsequent assisted reproductive technologies (ART) are never addressed in varicocelectomy outcome reports. We undertook a retrospective study of 59 men with varicoceles presenting to a male infertility clinic. Patients with varicoceles and normal seminal parameters were not offered surgical repair. We evaluated the difference in pregnancy outcome and seminal parameters after varicocelectomy surgery compared to the patients who did not have surgery. Approximately 50% of the patients underwent surgery and 37% from both groups underwent IVF. The mean post operative seminal parameters improved significantly and reached normal levels. The overall pregnancy rates (60%) for the two groups compared favorably to post varicocelectomy published series. Statistical significance in pregnancy rates was reached (p<0.02) when the non-IVF groups with partners greater than 35 years of age were considered. With IVF/ICSI ensuring egg penetration by the sperm, we nonetheless saw an improvement in egg fertilization rates and pregnancy rates in the whole surgical group. When the surgical and nonsurgical groups with female partners greater than 35 were compared, the pregnancy rate differential approached statistical significance.

Conclusion: We have shown that in the couple with male factor due to varicocele and with a partner greater than age 35 there appears to be a benefit in pregnancy rates after varicocelectomy, even when those couples undergo IVF.

TAIL TO HEAD RATIO (TH) OF FRESH AND FROZEN TESTICULAR SPERM AS A MEASURE OF VIABILITY J.L. Marmar, S.L. Corson, M.Gibbs, G.Huszar, Division of Urology, Robt. Wood Johnson Medical School at Camden, NJ, Fertility Testing Laboratory, Phila, PA, Dept. of OB-GYN, Yale University, New Haven, CT
When testicular sperm are extracted from biopsy material for ICSI, they are often nonmotile. The question that follows is whether these sperm are viable. Although vital stains may determine viability, they are undesirable for intracytoplasmic transfer. The hypomotile swelling test may determine viability, but this test may be difficult for cases with few available sperm. Recently, measurement of the TH ratio was used to determine sperm maturity based upon extrusion of the cytoplasmic droplet and CK values. Those sperm with long tails or at least a 10:1 ratio were most mature. In this report, we applied this simple measurement to fresh and frozen testicular sperm preserved for viability to determine whether TH could predict live sperm without further processing. Approximately 250 mgs of testicular tissue was obtained by open biopsy from 5 men undergoing vasectomy reversal. Each specimen was placed in a screw top vial with 0.5 ml Test Yolk Buffer (TYB). The fresh and post thaw specimen was compressed with a wooden applicator stick to remove the viable tissue and the remainder of the specimen was centrifuged and resuspended in 0.5 ml. A droplet was stained with SYBR-14 and propidium iodide. The viable sperm were green and the nonviable sperm were red. TH measurements were determined by a calibrated eye piece. A total of 116 sperm were counted, 51 viable and 65 nonviable. 82.3% viable sperm had TH>10, 24.6% nonviable sperm had TH>10. These differences were significant (chi square = 33, P=0.001). With TH ratio >10 and viability, sensitivity was 75.3%, specificity was 72.4%, and positive predictive value was 72.4% and likelihood ratio was 3.32. Thus, TH of fresh and frozen testicular sperm seems predictive of viability even among nonmotile sperm.

TESTICULAR AND EPIDIDYMAL SPERM OBTENTION: STILL AN EVOLUTIONAL PROCEDURE
Objectives: Intratesticular sperm injection (ICSI) has provided "fertility" to men with very low sperm count: Testicular and epididymal sperm are widely used, although there is no standardization of those procedures. This paper review the techniques used by us to get testicular and epididymal sperm from "nonviable" and the ICSI outcome with them.
Material and methods: During 1996, 24 ICSI cycles were done in 21 couples in whom the sperm was obtained from the testis (open biopsy or percutaneous needle aspiration) and epididymis (percutaneous needle aspiration).

Open biopsy or testicular sperm extraction (TESE): This procedure was done in 8 cycles in 8 couples and mean average age was 35 years. Mumps orchid, bilateral orchidopexy, anastomosis, obesity, inguinal varicose and failed varicocelectomy were the etiology of infertility. Average level of FSH was 32.8 (0 to 96, normal level <10 mU/mL). Two normal intraspermic biopsy under local anesthesia was done in all cases. Sperm was found in 5 cases and the fertilization rate after ICSI was 56.5%. An average of 2 motility was transferred to every woman, but 1 white there was no damage of the only egg fertilized. Two of them are pregnant now (27 and 20 weeks). In the other 3 couples in vitro fertilization was done with sperm freeze. One of these had a previous miscarriage and no sperm found. Their FSH levels were 5.2, 12.8 and 22.5. Two had normal total volume, which was reduced in one.

Percutaneous needle epididymal sperm aspiration (PESA): After June 96 it was done 6 cycles in 6 couples and mean average age was 35 years. One had one previous cycle with TESE. Bilateral orchidopexy, sperm maturation arrest, facial varicosecele and DES maternal ingestion were the etiology of sperm absence in the semen. Average level of FSH was 17.4 (4 to 21). Sperm aspiration was done under local anesthesia with a 20G syringe and a 25G needle. In every patient a postmortaly fragment was obtained. Sperm was found in 3 cases and the fertilization rate after ICSI was 50%. An average of 4 embryos was transferred to 4 women. In 1 case there was no fertilization of 2 oocyte. Injected. One of them is pregnant (16 weeks). Patient in whom no sperm was found had a positive previous biopsy 3 years before. His FSH level was 8.3.

Percutaneous needle epididymal sperm aspiration (PESA): It was done 10 cycles in 9 couples where mean average age was 43 years. Six patients had a vasectomy, 1 had a panadynical bilateral vasectomy, 1 had a nonmotile absence of vas deferens and 1 had a congenital absence of vas deferens (CVDV). Sperm was got with percutaneous aspiration of the epididymis with lue syringe and 25G needle, under local anestheia. Sperm was found in every case, but 1 with a normal vascuose and sperm was obtained with TESE. In man with CAVD the procedure was done twice and sperm was always found. In 2 cases when 8 millions of spermatozoon was obtained and part of the sample was frozen. ICSI was done in 8 cycle (there was no oocyte in 1 case and in the other the only oocyte was fragmented during ICSI) with a fertilization rate of 44.4%. Two woman are pregnant (37 and 20 weeks).

There were no complications in none of 3 procedures.

Conclusion: 1. TESE, TESA and PESA are useful and safe procedures. 2. FSH levels and total volume are not predictive data for finding sperm in testicular tissue of azoospermic men.

3. Previous testicular biopsy or aspiration could prevent unnecessary cytology induction in couples which man has sperm in the testis.

COMPARATIVE QUANTIFICATION OF THE MEMBRANE-ASSOCIATED UROKINASE PLASMINOGEN ACTIVATOR (uPA) ON THE EJACULATED SPERMATOZOA BETWEEN STERILE PATIENTS WITH ASTHENOSPERMIA AND INFERTILITY FERTILE MEN
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Semen were selected from 20 cases of sterile patients with asthenospermia and healthy fertile men respectively according to the standards of WHO. Semen was washed and kept with 0.1M Tris HCl buffer containing 1% Triton-100. To quantify uPA in the samples, polyclonal antibodies against human urokinase were employed by means of a sandwich ELISA. In lysates of spermatozoa, significantly lower levels of uPA were found in patients with asthenospermia [20.89±7.35 pg/ml (10⁶ cells)] as compared to healthy fertile men exhibiting normospermia [20.89±9.35 pg/ml (10⁶ cells)]. A positive correlation was observed between uPA quantity on spermatozoa and sperm viability [r=0.64, P<0.001], as well as with sperm motility [r=0.68, P<0.001]. Thus, it is inferred that membrane associated uPA on spermatozoa may be related to sperm motility and fertility.
STUDY ON LEVELS OF UROKINASE IN SEMEN PLASMA OF INFERTILE MEN WITH ABNORMAL LIQUEFACTION AND LOW SPERM MOTILITY

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The fact that urokinase exists in the human semen has been reported in many articles. Urokinase is a protein enzyme which is thought to play a role in semen liquefaction. Urokinase in semen plasma of 72 infertile men was examined by a sandwich ELISA. 72 semen samples were divided into three groups, normal sperm parameter group (30 cases), abnormal liquefaction group (21 cases) and asthenospermia group (22 cases). The results showed that levels of urokinase were 4600±1900 IU/L in normal sperm parameter group, 2720±1331 IU/L in abnormal liquefaction group, 2447±933 IU/L in asthenospermia group (P>0.01). There was no significant difference between abnormal liquefaction group and asthenospermia group (P>0.05). This suggests that changes of level of urokinase in semen plasma may influence sperm motility and semen liquefaction.
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