22nd Annual Meeting
February 22–25, 1997
Baltimore, Maryland
Program and Abstracts
**Friday, February 21**

12:00 NOON–11:00 PM  Executive Council Meeting (lunch and supper served) (Chesapeake Room A/B)

**Saturday, February 22**

<table>
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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:00–9:40 AM</td>
<td>Postgraduate Course (Constellation Ballroom A)</td>
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<tr>
<td>9:40–10:00 AM</td>
<td>Refreshment Break</td>
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<tr>
<td>10:00–12:00 NOON</td>
<td>Postgraduate Course (Constellation Ballroom A)</td>
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<tr>
<td>12:00–1:00 PM</td>
<td>Lunch (on your own)</td>
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<tr>
<td>1:00–2:40 PM</td>
<td>Postgraduate Course (Constellation Ballroom A)</td>
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<tr>
<td>2:40–3:00 PM</td>
<td>Refreshment Break</td>
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<tr>
<td>3:00–5:00 PM</td>
<td>Postgraduate Course (Constellation Ballroom A)</td>
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<tr>
<td>6:00–7:00 PM</td>
<td>Student Mixer (Maryland Suites-Baltimore Room)</td>
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<tr>
<td>7:00–9:00 PM</td>
<td>ASA Welcoming Reception (Atrium Lobby)</td>
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<tr>
<td>7:00–9:00 PM</td>
<td>Exhibits Open (Constellation Ballrooms E, F)</td>
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<tr>
<td>9:00–11:00 PM</td>
<td>Executive Council Meeting (Chesapeake Room A/B)</td>
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**Sunday, February 23**

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<th>Time</th>
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<tbody>
<tr>
<td>7:45–8:00 AM</td>
<td>Welcome and Opening Remarks (Constellation Ballroom A)</td>
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<tr>
<td>8:00–9:00 AM</td>
<td>Serono Lecture: “Genetics of Prostate Cancer” Patrick Walsh (Constellation Ballroom A)</td>
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<tr>
<td>9:00–10:00 AM</td>
<td>American Urological Association Lecture: “New Medical Treatments of Impotence” Irwin Goldstein (Constellation Ballroom A)</td>
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<tr>
<td>10:00–10:30 AM</td>
<td>Refreshment Break/Exhibits (Constellation Ballrooms E, F)</td>
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<tr>
<td>10:30–12:00 NOON</td>
<td>Oral Session I: “Genes and Male Reproduction” (Constellation Ballroom A)</td>
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<tr>
<td>12:00–1:30 PM</td>
<td>Lunch (on your own)</td>
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<tr>
<td>12:00–1:30 PM</td>
<td>Women in Andrology Luncheon (Chesapeake Room A/B)</td>
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<td>Business Meeting 12:00–12:30</td>
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<td>Speaker and Lunch 12:30–1:30</td>
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<tr>
<td>7:30–11:00 PM</td>
<td>Banquet (National Aquarium)</td>
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**Monday, February 24**

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<th>Time</th>
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<tr>
<td>7:00–8:00 AM</td>
<td>Past Presidents’ Breakfast (Pratt/Calvert Rooms)</td>
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<tr>
<td>8:00–9:00 AM</td>
<td>Oral Session III: “Sex Accessory Organs” (Constellation Ballroom A)</td>
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<tr>
<td>9:00–9:30 AM</td>
<td>ASA Lecture: “The Use and Abuse of Testicular Sperm As Viewed From a Thirty Year Experience With Epididymal Reconstructive Surgery” Robert Schoysman (Constellation Ballroom A)</td>
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<tr>
<td>9:30–10:00 AM</td>
<td>Refreshment Break/Exhibits (Constellation Ballrooms E, F)</td>
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<tr>
<td>10:00–11:00 AM</td>
<td>Pharmacie &amp; Upjohn Lecture: “Sex Chromosome Genes and Spermatogenesis” Colin Bishop (Constellation Ballroom A)</td>
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<tr>
<td>11:00–12:00 NOON</td>
<td>Buckeye State-of-the-Art Lecture: “Reproductive Rescue of Endangered Species” JoGayle Howard (Constellation Ballroom A)</td>
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<td>12:00–1:30 PM</td>
<td>Lunch (on your own)</td>
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**Tuesday, February 25**

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<th>Time</th>
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<tr>
<td>8:00–12:00 NOON</td>
<td>Andrology Laboratory Workshop (location to be announced)</td>
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<tr>
<td>8:00–9:30 AM</td>
<td>Oral Session IV: “Andrology and Assisted Reproductive Techniques” (Constellation Ballroom A)</td>
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<tr>
<td>9:30–10:00 AM</td>
<td>Refreshment Break/Exhibits (Constellation Ballrooms E, F)</td>
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<tr>
<td>10:00–12:00 NOON</td>
<td>Plenary Session: “Normal and Abnormal Prostate” William Nelson</td>
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<td>Martin Tenniswood</td>
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<td>ASA/National Medical Enterprises (NME) State-of-the-Art Lecture: “Gene Therapy of Prostate Cancer” Jonathan Simon (Constellation Ballroom A)</td>
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<td>Lunch (on your own)</td>
</tr>
<tr>
<td>1:00–5:00 PM</td>
<td>Andrology Laboratory Workshop (location to be announced)</td>
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American Society of Andrology

Executive Council

President .......................... Arnold M. Beeker, M.D.
Vice President ......................... Terry T. Turner, Ph.D.
Treasurer ............................. Terry R. Brown, Ph.D.
Secretary ............................. Rex A. Hess, Ph.D.
Past President ........................ Marie-Claire Orgebin-Crist, Ph.D.
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Laboratory Science Forum ........ Susan M. Tarchala, B.S.
Liaison ................................ Harris M. Nagler, M.D.
Local Arrangements 1997 ........... Terry R. Brown, Ph.D.

Journal of Andrology

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Journal of Andrology • Guggenheim 1711 • Mayo Clinic/Foundation • 200 First Street S.W. • Rochester, MN 55905
Office Hours: 8:00 a.m.–5:00 p.m. (Central) • Tel: 507/284-2423 • Fax: 507/284-2384 • E-mail: asa@mayo.edu

1997 Presidential Message

Welcome to the 22nd annual meeting of the American Society of Andrology! It is with a bittersweet feeling that many members travel to Baltimore for this meeting. We are reminded of the important role played in our society by Tom Chang, who is missed very much. Terry Brown capably assumed Tom’s role as local arrangements chairperson despite Terry's duty as our society’s treasurer. Terry's ability to spend time working on local arrangements was made easier by the willingness of Gail Prins to continue her duties as treasurer beyond the end of her term. Dorrie Lamb and Bob Oates are to be congratulated for arranging exciting programs for this Annual Meeting and Postgraduate Course, respectively.

Our society continues to prosper. Lonnie Russell is in the process of defining the role of the Publications Committee in various endeavors undertaken by our society. Significant improvements in the Journal of Andrology have been made by Ron Lewis and Don Tindall, and we anticipate further growth of the Journal under the new editorial direction soon to be provided by David Hamilton and Jon Pryor. Androlog continues to provide a busy forum concerning a variety of reproductive topics, and we are grateful to both Craig Niederberger and Andy Meacham for their continuing efforts with Androlog. Holland-Parlette Associates officially became our society’s business office on August 1, 1996. The dependable and efficient manner in which Carol Parlette and various members of Holland-Parlette Associates have conducted our business affairs justifies the recommendation of their firm by the Finance Committee, which is chaired by Rebecca Sokol.

Each participant in this year's meeting is encouraged to attend the banquet, which will be held in the National Aquarium. The format for the evening will allow ample time both for touring this interesting facility and for interacting with colleagues, while leisurely having supper at various stations along the way.

We appreciate the valued support of our organization by the various industrial contributors and exhibitors. I am particularly indebted to the Jewish Hospital HealthCare Services in Louisville, Kentucky both for administrative help with my presidential duties and for generous support of this year’s annual meeting.

Finally, I want to thank the membership for the opportunity I have had to serve as president of our society. I feel particularly honored to be the first urologist to serve in this capacity. I have enjoyed working with the officers, Council members and committee chairs. Our society is successful because of the unusual dedication of so many members.

Arnold M. Beeker
President
Past Presidents

1975–1977 ...................... Emil Steinberger
1977–1978 ...................... Don W. Fawcett
1978–1979 ...................... C. Alvin Paulsen
1980–1981 ...................... Philip Troen
1983–1984 ...................... Andrzej Bartke
1984–1985 ...................... Rudi Ansbacher
1985–1986 ...................... Anna Steinberger
1987–1988 ...................... Larry L. Ewing
1988–1989 ...................... C. Wayne Bardin
1989–1990 ...................... Rupert Aman
1990–1991 ...................... Howard Nankin
1992–1993 ...................... Ronald S. Swerdloff
1993–1994 ...................... Bernard Robaire
1994–1995 ...................... Glenn R. Cunningham

General Information

Transportation and Travel Arrangements

By Car: From Washington, D.C. & Virginia: Take I-95 North to Baltimore. Exit 53 (395) N Downtown/Inner Harbor. 395 becomes Howard Street, continue 2 blocks and then turn right on Pratt Street. Follow 4 blocks and turn right at Light Street. The Hyatt is ½ block on the right.

From Philadelphia/New Jersey/New York: Take 95 South through the Fort McHenry Tunnel and continue on 95 to Exit 53 (395) N Downtown/Inner Harbor.

From Frederick/West Virginia/Western Maryland: Take 70 East to 695 South to Glen Burnie. Follow 695 to Exit 11 (95) North to Baltimore. Continue on 95 North to Exit 53 (395) N Downtown/Inner Harbor.

From Pittsburgh, PA: Take Rt. 76 to Rt. 70 East. Follow above directions from Frederick.

From Annapolis, MD: Take Rt. 50 West to I-97 North which becomes Rt. 3 North to 695 West. Follow 695 West to Exit 11 (85) North to Baltimore. Continue on 95 North to Exit 53 (395) N Downtown/Inner Harbor.

From Harrisburg, PA: Take I-83 South to Baltimore. Follow 83 South to end and this becomes President St. Continue 2 blocks and turn right on Lombard St. Follow 7 blocks to Light Street and turn left. The Hyatt is ½ blocks on the right.

By Airplane and Car: For those wanting to have personal transportation in the Baltimore Metro area, all major car rental firms are represented at the Baltimore-Washington International Airport.

For travel from the airport to the hotel, exit the airport area via I-195W and follow signs to I-95N to Baltimore. From I-95, take exit 53 for I-395N for Downtown/Inner Harbor. I-395 becomes Howard Street at the first traffic signal. Continue two blocks and turn right onto Pratt Street. Follow 4 blocks and turn right onto Light Street. The Hyatt is ½ block on the right.

On-Site Registration

On the Inner Harbor
Hyatt Regency Hotel Baltimore
On the Inner Harbor
300 Light Street
Baltimore, MD 21202
Tel: (410) 528-1234
Fax: (410) 385-1170

Mail-In Registration
American Society of Andrology
74 New Montgomery, Suite 230
San Francisco, CA 94105
Tel: (415) 764-4823
Fax: (415) 764-4915
E-mail: 105037.1120@compuserve.com

Exhibits
Constellation Ballrooms E and F
Saturday, February 22: 7:00 PM–9:00 PM
Sunday, February 23: 9:00 AM–5:00 PM
Monday, February 24: 9:00 AM–5:00 PM

Slide Preview and Press Room
The Charles Room will be open during the meeting hours (7:30 AM to 6:00 PM) from Saturday, February 22 to Tuesday, February 25.

Miscellaneous Meetings
The Calvert or Pratt Rooms can be reserved for committee meetings or small group meetings (please contact Sarah Morisseau in the ASA Business Office).

Smoking Regulations
Smoking is not permitted in the meeting rooms or in the exhibit and poster areas.
By Taxi or Airport Shuttle Service: Taxi fare from BWI airport to the meeting hotel is approximately $20. The Airport Super Shuttle service is available from the baggage claim area. Fares are $10 one-way or $15 round-trip.

Travel Arrangements and Hotel Reservations:
Arrangements for special airfares can be made by contacting Ann Adams at Buck Rogers Travel [Tel: (800) 580-2472 or Fax: (915) 581-8172]. Mention that you are with the American Society of Andrology.

Hotel Reservations

Hotel reservations should be made using the Hotel Reservation form in the registration package, or by calling the hotel directly at (410) 528-1234 or Hyatt Reservations at (800) 233-1234. To ensure receiving the special meeting rates, you must identify the group name when making reservations.

Rates and room availability can only be guaranteed for reservations received by January 20, 1997.

Message from the Local Arrangements Committee

Welcome to Baltimore's Inner Harbor in February!! Remember, spring is just around the corner. This, the 22nd Annual Meeting of the ASA, promises to provide a stimulating and challenging scientific program for all attendees. Robert Oates and the Postgraduate Course Committee have put together a course on the cutting edge of Technologies and Genetic Advances in Animal and Human Reproduction. Dolores Lamb and the Annual Meeting Program Committee have designed an exciting daily scientific venue comprised of Plenary sessions, oral presentations and posters. Our Corporate Exhibitors will be displaying and demonstrating the latest technologies and instrumentation in andrology. For our premier social event, we have procured the main pavilion of Baltimore's National Aquarium for a private viewing of the exhibits, complete with a fish feeding demonstration and a visit to the tropical rain forest, which will be combined with cocktails, hors d'oeuvres, and a sumptuous buffet dinner. The Student Colloquium will be highlighted by the provocative and entertaining multi-media presentation of Donald Coffey entitled "On Human Destiny".

The Hyatt Regency Hotel is located in the center of downtown Baltimore overlooking the scenic Inner Harbor and within walking distance of the National Aquarium, the Maryland Science Center, the Morris Mechanic Theatre, the Baltimore Arena for indoor sports events, and the shops and restaurants surrounding the harbor area. The Meyerhoff Symphony Hall, home to the internationally acclaimed Baltimore Symphony Orchestra, is only a short cab ride away. The University of Maryland Medical Center is within several blocks of the hotel and the Johns Hopkins Medical Institutions can be reached by Metro from the downtown Charles Center station. We will have a list of current events, restaurants and directions for travel available at the registration desk. We hope you will enjoy your visit to Baltimore and please let us know how we can help you during your stay.

Terry Brown, Ph.D.
Local Arrangements Chairperson

Student Information

Colloquium
Monday, February 24, 1997 from 7:00–9:00 PM in the Constellation Ballroom.
A multi-media presentation entitled "On Human Destiny"

Speaker
Donald Coffey

Colloquium Sponsor
California Cryobank, Inc.

Mixer
Saturday, February 22 from 6:00–7:00 PM in the Maryland Suites-Baltimore Room.

Placement Service: A Placement Service for candidates and employers is available. The Placement Service Board will be near the Registration Desk. To register prior to the meeting, contact: Dr. Pasquale Patrizio, Director, Male Infertility Service, Division of Human Reproduction, Department of Obstetrics & Gynecology, University of Pennsylvania–School of Medicine, 106 Dulles Building, 3400 Spruce Street, Philadelphia, PA 19104 (tel: 215-662-2975, fax: 215-349-5512).

Student Awards: A New Investigator Award of $500 and five Student Merit Awards of $100 will be presented to students on the basis of their presentations at the Annual Meeting. The winners will be selected by the Awards Committee.

Local Arrangements Committee: Partha Banerjee, Angela Brodie, Terry Brown (Chairperson), Adrian Dobs, Brad Lerner, Deborah Ricker, Kevin Whaley, and Barry Zirkin.
Message from the Program Chairperson

The American Society of Andrology consists of a diverse group of basic scientists, andrologists, urologists, gynecologists, veterinary scientists and endocrinologists—all with a common interest in Male Reproduction. This year’s program committee has attempted to include speakers that will be of general interest to both the basic scientist and the clinician members of our society.

The Serano Lecturer is Dr. Patrick Walsh of Johns Hopkins University, who will discuss “The Genetics of Prostate Cancer”. Dr. Walsh is a recognized leader in the field of both the clinical treatment and the molecular basis of prostate cancer. Identification of specific genes involved in the acquisition or progression of this common urologic tumor in men will provide important insights into the etiology of prostate cancer. In addition, the American Urological Association will sponsor a lecture honoring a prominent urologist. Dr. Irwin Goldstein, Boston University School of Medicine, has been selected to discuss “New Medical Treatments of Impotence”. Dr. Goldstein is an expert in the basic science and clinical treatment of erectile dysfunction. He has also contributed to our understanding of erectile physiology and vasculogenic impotence.

The Sunday symposium is focused upon the regulation of testicular growth and function. Dr. Patricia Morris, from the Population Council of Rockefeller University, will discuss cytokine signal transduction in the testis via the JAK-STAT pathway. Testicular cytokines and growth factors activate tyrosine phosphorylation of STAT proteins, leading to nuclear translocation of these transcription factors. Specific receptors thereby induce transcriptional activation of target genes in a cell- and age-specific manner. Targeted deletion of genes and transgenic technology has provided important tools for the identification of key regulatory genes in male reproduction. Dr. Martin Matzuk, Baylor College of Medicine, has used these techniques to evaluate the actions of gonadotropins, inhibins and activins and various other hormones on reproduction.

Dr. Robert Schoysman (Brussels, Belgium) will share his thoughts on the use of testicular sperm in assisted reproductive techniques. This talk will be based on his perspective of having performed epididymal reconstructive surgery for over thirty years.

The Pharmacia & Upjohn Lecture, by Dr. Colin Bishop (Baylor College of Medicine), will focus on an area of key importance in our understanding of male sexual development and spermatogenesis, namely the identification of genes on the sex chromosomes. The Buckeye State-of-the-Art Lecture will feature Dr. JoGayle Howard on the “Reproductive Rescue of Endangered Species”. This is a significant problem facing our world today.

A second symposium will focus on a very controversial area in male reproductive medicine, namely the role of estrogens in the male. Estrogens have been implicated in the normal function of the testis as described by Dr. E. Mitchell Eddy and in hormonal carcinogenesis as discussed by Dr. John McLachlan. Most recently, environmental estrogens have been implicated in purported declines of various aspects of human reproductive health. Dr. Stephen Safe will report on a variety of naturally occurring and synthetic estrogens and anti-estrogens and address the potential of these compounds to act as hormonal disruptors.

The meeting will close with a symposium on the normal and abnormal prostate. The ASA/National Medical Enterprises Lecture will be given by Dr. Jonathan Simon on “Gene Therapy of Prostate Cancer”. This area of research is particularly timely and addresses a key area of research that may provide a potential new therapy for prostate cancer. New markers of prostatic disease can provide important diagnostic tools to the clinician. Dr. Bill Nelson will discuss his most recent studies and provide attendees with some insight into whether these new markers help with the diagnosis of human benign prostatic hyperplasia and prostate cancer. Dr. Martin Tenniswood will conclude the session with a lecture on androgen regulated prostate gene expression. This is an area of key interest to many of the andrologists and endocrinologists.

Dolores J. Lamb, Ph.D.
Program Chairperson

Continuing Medical Education Credit

The University of Minnesota is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

Annual Meeting:
The University designates this continuing education activity as Category 1 of the Physician's Recognition Award of the American Medical Association. One credit hour may be claimed for each hour of participation up to a maximum of 17.0 hours.

Post Graduate Course:
The University of Minnesota designates this continuing medical education activity for 7.0 hours in Category 1 of the Physician's Recognition Award of the American Medical Association.
Educational Objectives for CME Credit

ASA Annual Meeting

By attending the 1997 Annual Meeting, the participants will be able to:
* Discuss the genetics of prostate cancer;
* Describe new medical treatments for impotence;
* Define new advances in the regulation of testicular growth and function;
* Explain sex chromosome genes and spermatogenesis;
* Describe techniques used for reproductive rescue of endangered species;
* Summarize the role of estrogen in normal male reproductive function, hormonal carcinogenesis, and reproductive toxicology;
* List the latest experiments on normal and abnormal prostate.

ASA Postgraduate Course

Following this course entitled, "Technological and Genetic Advances in Animal Reproduction," the participants will be able to:
* Describe the need for and techniques involved in animal contraception;
* Describe why certain species are experiencing a decline in their reproductive capability;
* Understand the advanced reproductive techniques that are used in veterinary medicine;
* Describe how gene transfer in animals works;
* Describe the genetic mechanisms involved in testicular differentiation and spermatogenesis;
* Understand the molecular and genetic aspects of fertilization and early embryo development, i.e., the role of sperm;
* Gain an appreciation for the concerns regarding the use of testicular spermatozoa and spermatids.

1997 American Society of Andrology Postgraduate Course
February 22, 1997, Hyatt Regency
Baltimore, Maryland

"Technological and Genetic Advances in Animal Reproduction"

Course Director: Robert Oates

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<th>Time</th>
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<td>8:00-8:10 AM</td>
<td>Welcome and Introduction Robert Oates</td>
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<td>8:10-8:50 AM</td>
<td>&quot;Contraceptive Techniques in Endangered Animal Species—Why do We Need Them?&quot; Linda Munson</td>
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<td>8:50-9:30 AM</td>
<td>&quot;Reproductive Decline in Animals: Genetic Aspects&quot; Steven O'Brien</td>
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<td>9:30-9:50 AM</td>
<td>Refreshment Break</td>
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<td>9:50-10:30 AM</td>
<td>&quot;Advanced Reproductive Techniques in Domestic Animals&quot; Rupert Amann</td>
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<tr>
<td>10:30-11:10 AM</td>
<td>&quot;Gene Transfer in Animals&quot; Robert Wall</td>
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Questions and Answers
All Morning Speakers
Lunch (on your own)
Introduction Robert Oates
"Testicular Differentiation From the Primitive Gonad: Genetic Control and Morphological Aspects" Patricia Donahoe
Questions
"Genetic Mechanisms of Azoo-spermia" Renee Reijo
Questions
"Advantages of the Human Model in Reproductive Genetic Studies" Renee Reijo
Questions
Refreshment Break
"The Inheritence of the Sperm Centrosome, the Cell's Microtubule Organizing Center, During Human Fertilization" Cal Simerly
Questions
2:45–3:05
"Molecular Reconstitution of the Human Sperm Centrosome: Implications for Diagnosing New Forms of Male Infertility"
Cal Simerly
Questions

3:10–3:30 PM
"Paternal Contribution to Early Embryo Development and Growth-Importance of Genomic Imprinting"
Benjamin Tycko
Questions

3:35–3:55 PM
"Genomic Imprinting: When it Occurs and Clinical Syndromes Resulting from Imprinting Errors"
Benjamin Tycko
Questions

4:00–4:25 PM
"Clinical Experience and Ethical Considerations of the Use of Spermatis for IVF/ICSI"
Robert Schoysman
Questions

4:30–5:00 PM
Questions for all speakers

Serono Lecture
Sunday, February 23, 8:00 AM
Patrick Walsh, Johns Hopkins University School of Medicine, Baltimore, MD, "Genetics of Prostate Cancer"

American Urological Association (AUA) Lecture
Sunday, February 23, 9:00 AM
Irwin Goldstein, Boston University School of Medicine, Boston, MA, “New Medical Treatments of Impotence”

ASA Lecture
Monday, February 24, 9:00 AM
Robert Schoysman, Brussels, Belgium, “The Use and Abuse of Testicular Sperm As Viewed From a Thirty Year Experience With Epididymal Reconstructive Surgery”

Buckeye State-of-the-Art Lecture
Monday, February 24, 11:00 AM

ASA/National Medical Enterprises (NME) State-of-the-Art Lecture
Tuesday, February 25, 10:00 AM
Jonathan Simon, Johns Hopkins University School of Medicine, Baltimore, MD, “Gene Therapy of Prostate Cancer”

Symposium I—
“Regulation of Testicular Growth and Function”

Sunday, February 23, 1:30 PM

Symposium II—
“Estrogen in the Male: Hormonal Carcinogenesis, Reproductive Toxicology, and Role in Normal Reproductive Function”

Monday, February 24, 1:30 PM

Pharmacia & Upjohn Lecture
Monday, February 24, 10:00 AM
Colin Bishop, Baylor College of Medicine, Houston, Texas, “Sex Chromosome Genes and Spermatogenesis”

Program Committee
D. Lamb (Chair), M. Anderson, W. Hellstrom, B. Hinton, R. Oates, K. Roberts, L. Rodriguez-Rigau, D. Tindall, A. Belker

Abstract Review Committee
D. Lamb (Chair), S. Benoff, G. Centola, W. Hellstrom, B. Hinton, R. Oates, K. Roberts, L. Rodriguez-Rigau, D. Tindall, A. Belker
Patrick C. Walsh, M.D. is the David Hall McConnell Professor and Director of the Department of Urology at the Johns Hopkins University School of Medicine and the Urologist-in-Chief of the James Buchanan Brady Urological Institute of the Johns Hopkins Hospital, Baltimore, Maryland. Dr. Walsh received his M.D. in 1964 from Case Western Reserve University. From 1964 through 1967 he was a resident in surgery and pediatric surgery at the Peter Bent Brigham Hospital and Boston Children’s Hospital. From 1967 through 1971 he was a urology resident at UCLA. In 1974 Dr. Walsh became the Director and Chairman of Urology at Johns Hopkins where he has worked over the past 22 years perfecting the surgical management of prostate cancer. He is the Editor-in-Chief of *Campbell’s Textbook of Urology* published by W.B. Saunders and is a member of the Institute of Medicine of the National Academy of Sciences. In June, 1996 Dr. Walsh received the Charles F. Kettering Medal from the General Motors Cancer Research Foundation for “The Most Outstanding Recent Contributions to the Diagnosis or Treatment of Cancer”. This award was shared with Dr. Malcolm A. Bagshaw, Professor Emeritus of Radiation Oncology, Stanford University. Dr. Walsh has received numerous other awards and citations. He has served on the Editorial Boards of journals such as the *Journal of Urology, Fertility and Sterility, the Journal of Andrology* and has edited many important Urology textbooks. Among his major original scientific contributions, he was the first to describe the 5a-reductase enzyme deficiency. This work established the embryologic and physiologic consequences of dihydrotestosterone deficiency in man. He established the rat as a model to study penile erection. He showed experimentally that cavernous nerve grafts could be used to restore erectile function. One study focused upon the identification of an autosomal dominant inheritance of Peyronie’s disease. He is well known for his studies on the prostate. Dr. Walsh demonstrated that benign prostatic hyperplasia could be induced in the dog by hormonal manipulation and provided the first scientific evidence that estrogen may synergize with androgen in the induction of BPH, as well as the reversible influence of androgen deprivation on BPH. Notably, he has developed a nerve-sparing method of radical prostatectomy that preserves sexual function. Most recently, Dr. Walsh was the first to recognize and characterize hereditary prostate cancer. This may lead to insight into the genetic mechanisms responsible for prostate cancer.
the Benchmark Papers in Human Physiology Series, described him as follows, "A true Renaissance man, Dr. Setchell is fluent in several languages, played bassoon and contrabassoon for many years in the Cambridge Philharmonic Society Orchestra and is a student of both general and medical history—in short, an ideal individual to guide us through the significant history of male reproductive biology." Dr. Setchell has translated the entire works of Regnier de Graaf on male and female reproduction (published by the Journal of Reproduction & Fertility as Supplement 17). He has also translated the original paper by Sertoli, describing the somatic supporting cells of the seminiferous tubules. Dr. Setchell has served on, or currently serves as, a member of the Council of Management for Biology of Reproduction, Council of Management for the Journal of Reproduction and Fertility, the Editorial Boards of Molecular & Cellular Endocrinology, Reproduction, Fertility and Development, and Reproduction, Nutrition and Development. Dr. Setchell is recognized for having and trying out new ideas that may not have been accepted science at the time of their investigation. He is credited for having imaginative insight into male reproductive physiology and has applied careful science to further our understanding of testicular function. He has supervised at least twenty Ph.D. candidates for Cambridge and Adelaide Universities as well as the National Council for Academic Awards of the United Kingdom. For his unique and original contributions to andrology that are considered the basis for our understanding of testicular physiology, his active enthusiasm for research and teaching, as well as his support of young scientists entering the study of andrology, the Society is delighted to award Dr. Brian P. Setchell with the 1997 Distinguished Andrologist Award.

Young Andrologists

<table>
<thead>
<tr>
<th>Year</th>
<th>Name</th>
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</thead>
<tbody>
<tr>
<td>1976</td>
<td>Roy O. Greep</td>
</tr>
<tr>
<td>1977</td>
<td>Roberto E. Mancini</td>
</tr>
<tr>
<td>1978</td>
<td>Robert J. Hotchkiss</td>
</tr>
<tr>
<td>1979</td>
<td>Thaddeus Mann</td>
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<tr>
<td>1980</td>
<td>John MacLeod</td>
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<tr>
<td>1981</td>
<td>Alexander Albert</td>
</tr>
<tr>
<td>1982</td>
<td>Eugenia Rosemberg</td>
</tr>
<tr>
<td>1983</td>
<td>Kristen B. D. Eik-Nes</td>
</tr>
<tr>
<td>1984</td>
<td>Mortimer B. Lipsett</td>
</tr>
<tr>
<td>1985</td>
<td>Robert H. Foote</td>
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<tr>
<td>1986</td>
<td>Alfred D. Jost</td>
</tr>
<tr>
<td>1987</td>
<td>Emil Steinberger</td>
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<tr>
<td>1988</td>
<td>Yves W. Clermont</td>
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<tr>
<td>1989</td>
<td>C. Alvin Paulsen</td>
</tr>
<tr>
<td>1990</td>
<td>Marie-Claire Ogerbin-Crist</td>
</tr>
<tr>
<td>1991</td>
<td>Philip Troen</td>
</tr>
<tr>
<td>1992</td>
<td>C. Wayne Bardin</td>
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<td>1993</td>
<td>Anna Steinberger</td>
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<tr>
<td>1994</td>
<td>Richard J. Sherins</td>
</tr>
<tr>
<td>1995</td>
<td>Rupert P. Amann</td>
</tr>
<tr>
<td>1996</td>
<td>J. Michael Bedford</td>
</tr>
</tbody>
</table>

Young Andrologists Award

Gail A. Cornwall is the recipient of the 1997 Young Andrologist Award. Dr. Cornwall received her B.S. and M.S. from Brigham Young University and her Ph.D. in Reproductive Biology from The Johns Hopkins University School of Hygiene and Public Health in 1988. She was an NIH NRSA fellow at Vanderbilt University and subsequently Research Assistant Professor at Vanderbilt in the Department of Cell Biology from 1991–94. Dr. Cornwall travelled to Lubbock, Texas in 1994, where she currently serves in an announcement as Assistant Professor in the Department of Cell Biology and Biochemistry at the Texas Tech University Health Sciences Center. Dr. Cornwall has worked extensively in the area of sperm maturation and studied the acquisition of sperm motility that occurs in the epididymis. Her pre-doctoral work at The Johns Hopkins University was performed under the direction of Dr. Thomas S.K. Chang and Dr. Larry Ewing. She continues to investigate epididymal biology, using molecular techniques that have allowed the isolation of a novel and reproductive-specific gene, CRES (cystatin-related epididymal-spermatogenetic). Her work has involved not only isolating this gene but characterizing its expression pattern. The highly transient nature of protein expression in the male reproductive tract suggests that CRES may play a specialized role in epididymal and testicular function. Ongoing NIH support allows her to further evaluate the role of this important, highly conserved gene, CRES, in the process of sperm development and maturation. Dr. Cornwall has received numerous student research awards from the American Society of Andrology, and she has been an invited presenter at ASA Postgraduate Courses, the Gordon Research Conference, and recognized by an invitation to write a review on epididymal-specific gene expression in the Journal of Andrology. She serves as a member of the Editorial Board of the Journal of Andrology, member of the Student Affairs Committee, Program Committee and Long-Range Planning Committees for the American Society of Andrology. Dr. Cornwall serves as an excellent role model for young scientists, especially women in andrology. Her professional, yet warm interactions with colleagues and students are remarked upon universally by any scientist who has had the privilege to work with her. Her scientific presentations are always clear and precise. For her research, her character, and continued contributions to andrology, the American Society of Andrology is delighted to award Dr. Gail A. Cornwall with the 1997 Young Andrologist Award.

Young Andrologists

<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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<tr>
<td>1984</td>
<td>Lonnie D. Russell</td>
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<td>1985</td>
<td>Bruce D. Schanbacher</td>
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<tr>
<td>1986</td>
<td>Stephen J. Winter</td>
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<tr>
<td>1987</td>
<td>Ilpo T. Huhtaniemi</td>
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<tr>
<td>1988</td>
<td>Larry Johnson</td>
</tr>
<tr>
<td>1989</td>
<td>Barry T. Hinton</td>
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<tr>
<td>1990</td>
<td>Luis Rodriguez-Rigau</td>
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<td>1991</td>
<td>Patricia M. Saling</td>
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<tr>
<td>1992</td>
<td>Gary R. Klinefelter</td>
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<td>1993</td>
<td>Robert Chapin</td>
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<tr>
<td>1994</td>
<td>Wayne J.G. Hellstrom</td>
</tr>
<tr>
<td>1995</td>
<td>Christopher J. De Jonge</td>
</tr>
<tr>
<td>1996</td>
<td>Paul S. Cooke</td>
</tr>
</tbody>
</table>

Sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.
Distinguished Service Award

Marie-Claire Orgebin-Crist is the recipient of the 1997 Distinguished Service Award. Dr. Orgebin-Crist received her License of Natural Sciences and License of Biology from the University of Paris in 1957 and her Doctorate of Sciences from the University of Lyon, in France, in 1961. After post-doctoral work in the Department of Biochemistry, Faculty of Medicine in Paris as well as the National Center for Scientific Research in Paris, she served as a Research Associate at The Population Council in New York in 1962–63 and went to Vanderbilt University, Nashville, Tennessee in 1963. Dr. Orgebin-Crist has remained at Vanderbilt since that time, starting as a Research Associate in 1963, was promoted to Research Instructor in 1964, to the level of Assistant Professor in 1966, and to Associate Professor in 1970. Dr. Orgebin-Crist was awarded full Professorship and named as occupant of the Lucius E. Burch Chair of Reproductive Biology and Director of the Division as well as Center for Reproductive Biology Research in 1973. She was also named as a Professor in the Department of Cell Biology in 1975. Dr. Orgebin-Crist has had a distinguished research career, with invaluable contributions to our understanding of the function and relevance of the epididymis. She received the Distinguished Andrologist Award from the American Society of Andrology in 1990, and served as the second editor of the Journal of Andrology. She was the Serono Lecturer in 1995 and is currently the past President of the Society, having served as President from 1995–96 and Vice President from 1994–95. Dr. Orgebin-Crist was recipient of the Presidential Award of ASA in 1982, made an honorary member of the Canadian Fertility & Andrology Society in 1985, and received the Distinguished Scientist Award from the American Society for Reproductive Medicine (formerly known as the American Fertility Society) in 1996. She has served as chair of the ASA Nominations Committee (1981–83), Program Committee (1977–78), and International Liaison Committee (1993–95), as well as the Executive Council from 1978–81. In addition, Dr. Orgebin-Crist has served on Publications (1975–76), Finance (1980–81), Membership (1978–79), Nominations (1975–76) and Program Committees (1980–81) for the ASA. Dr. Orgebin-Crist has also been very active in the International Society of Andrology, currently serving on both the Executive Council (since 1989) and Program Organizing Committees (since 1993). Her involvement with the Society for the Study of Reproduction has also been extensive. She has played an important role for the National Institutes of Health, serving as member of the Contract Review Committee for the Center for Population Research, as a member of the Population Research Committee for NICHD, as a member of the Reproductive Biology Study Section, as a Key Consultant to the NICHD Five Year Research Plan in 1980, as well as a member of the Five Year Research Plan for the Reproductive Sciences Branch of the Center for Population Research, NICHD, in 1996. Dr. Orgebin-Crist has also served the World Health Organization, as a member of the task force on methods for the Regulation of Male Fertility (1972–75) and ad hoc member of the Review Committee on Resources for Research (1985–90). She has served or continues to serve as a member of the Editorial Boards of Andrologia, Biology of Reproduction, Journal of Andrology, and International Journal of Andrology, as well as Associate Editor for Gamete Research, Molecular Reproduction & Development and Revue Maghrebine d’Endocrinologie-Diabete et Reproduction. One of her greatest contributions to andrology has been in the training and support of numerous Postdoctoral Fellows, Visiting Scientists, and Ph.D. students. Many of her former students have gone on to be active contributors to the American Society of Andrology and leaders in the field of andrology. Not only as she made extensive contributions to andrology service, training and research, but Dr. Orgebin-Crist has also fostered new scientists by making them feel welcome into the family of andrologists. For her active service, enthusiastic support and extensive involvement in andrology, the Society is delighted to award Dr. Marie-Claire Orgebin-Crist with the Distinguished Service Award.

Distinguished Service Award Recipients

1994 C. Alvin Paulsen
1995 Andrzej Bartke
1996 Philip Troen

New Investigator Award

Recipient will be announced at the Awards Ceremony on Monday, February 24, 1997 at 3:30 PM.

New Investigator Award Recipients

1983 Thomas T. Tarter
1984 Peter S. Albertson
1986 Mark A. Hadley
1987 Peter Grosser
1988 Stuart E. Ravnik
1989 Tracy L. Rankin
1990 Donna O. Bunch
1991 Robert Viger
1992 John Kirby
1993 Michael A. Palladino
1994 Linda R. Johnson
1995 Mehdi A. Akhondi
1996 Wei Gu
Daniel B. Rudolph

Sponsored by the West Michigan Reproductive Institute, P.C.
The American Society of Andrology wishes to thank the following organizations for their generous support:

**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

National Medical Enterprises
West Michigan Reproductive Institute, P.C.

**Sustaining Sponsor**

A minimum $500 contribution to ASA for five or more years

Hamilton Thorne Research
Pharmacia & Upjohn
SeroN Laboratories, Inc.
Texas Institute for Reproductive Medicine and Endocrinology, P.A.

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**Supporters of the 1997 Annual Meeting & Postgraduate Course**

Abbott Laboratories
Alza Pharmaceuticals
American Urological Association
California Cryobank, Inc.
Genetics & IVF Institute
Jewish Hospital HealthCare Services, Louisville, Kentucky
Merck & Co., Inc.
Pfizer U.S. Pharmaceuticals Group
Anna Steinberger, Ph.D.

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**Future Meetings**

**March 28–31, 1998**

23rd Annual Meeting of the American Society of Andrology and Postgraduate Course
Hyatt Regency Long Beach, Long Beach, California, USA
Contact: ASA Executive Offices
74 New Montgomery, Suite 230, San Francisco, California 94105
phone (415) 764-4823; fax (415) 764-4915; e-mail 105037.1120@compuserve.com.

**April 9–12 or April 16–19, 1999**

24th Annual Meeting of the American Society of Andrology and Postgraduate Course
Chicago, Illinois, USA
Contact: ASA Executive Offices
74 New Montgomery, Suite 230, San Francisco, California 94105
phone (415) 764-4823; fax (415) 764-4915; e-mail 105037.1120@compuserve.com.
Women in Andrology Luncheon

“Successful Mentoring”

Chairs: Donna Vogel, Ph.D. and Dolores Lamb, Ph.D.
Speaker: Anna Steinberger, Ph.D.
Date: Sunday, February 23, 1997
Time: 12:00–12:30 PM (Business Meeting)
       12:30–1:30 PM (Speaker and Lunch)
Place: Chesapeake Room A/B

(Registration fee: $22 (includes lunch)—see Registration Form)

Laboratory Science Forum

“The Basics of Gamete Cryopreservation”

Chair: Susan Tarchala, B.S., T.S.
Speaker: Grace Centola, Ph.D.
University of Rochester
Date: Monday, February 24, 1997
Time: 12:00–1:30 PM
Place: Maryland Suites-Columbia Room

(Lunch available for $20—See Registration Form)

Participants will learn about donor selection and screening, cryopreservation, client depositor versus donor and receive an update on oocyte and embryo cryopreservation and donation.

Student Colloquium

(Sponsored by: California Cryobank, Inc., Los Angeles, CA)

“On Human Destiny”

Chair: Grace Centola, Ph.D.
Speaker: Donald Coffey, Ph.D.
        Johns Hopkins School of Medicine
Date: Monday, February 24, 1997
Time: 7:00–8:30 PM
Place: Maryland Suites-Annopolis/Baltimore Rooms
Andrology Laboratory Workshop
(Hosted by: 1997 Annual Andrology Laboratories Committee (ALC)
in Conjunction with the American Society of Andrology)

“Moving Beyond Boundaries: Clinical Andrology in the 21st Century”

Director: Christopher De Jonge, Ph.D., H.C.L.D.

Chairs: Christopher Barratt, Ph.D., Sheffield, UK
        Pasquale Patrizio, M.D., Philadelphia, PA

Topics:
1. “Introduction to Molecular Biology: The Language & Methods”
2. “Recombinant Protein Technology in the Andrology Lab”
4. “Clinical Application of Molecular Biology: Cystic Fibrosis Mutations, Y-Chromosome Deletions and the Future”
5. “DNA Breakage: Why is it Important for the Andrologist?”

Date: Tuesday, February 25, 1997
Time: 8:00 AM–5:00 PM
Place: Location to be announced

Course Description
Molecular genetic techniques are greatly advancing our understanding of many events taking place during the reproductive process. However, for many, the language and the methodologies of these techniques remain obscure. This course is specifically designed to provide the key background information to facilitate the understanding, the current use and the potential applications of molecular biology techniques in the field of andrology. The course is structured in a morning session where the participants will be introduced to the basic language and to the methodologies currently in use (i.e., PCR for DNA and RNA cloning, recombinant DNA, testis libraries, in situ hybridization, etc.). The afternoon session will cover the clinical application of these techniques. Finally, to enable attendees to verify their understanding of the topics covered, the participants will be organized in small groups and assigned each with a specific genetic problem to be solved with the help of the faculty.

Course Objectives
After attending this course, the participant will be able to:
1. Understand the language and the methodologies used in molecular biology;
2. Describe the application, power and short falls of each technique;
3. Interpret current literature in genetic andrology;
4. Identify new areas of research in both basic science and clinical andrology.

The course is specifically designed for andrologists, whether clinicians or laboratory directors, biologists, technicians, researchers and students.

Registration fee is $125 (ASA Student Member) or $150 (Student Nonmember) and $175 (ASA Member) or $200 (Nonmember), with a late fee of $25 after January 20, 1997.
SOCIAL/MEETING EVENTS AT A GLANCE

Friday, February 21, 1997

12:00 NOON–11:00 PM  Executive Council Meeting (Chesapeake Room A/B)

Saturday, February 22, 1997

8:00 AM–5:00 PM  Postgraduate Course (Constellation Ballroom A)
6:00 PM–7:00 PM  Student Mixer (Maryland Suites-Baltimore Room)
7:00 PM–9:00 PM  Welcoming Reception (Atrium Lobby)
                 Exhibits Open (Constellation Ballrooms E, F)
9:00 PM–11:00 PM  Executive Council Meeting (Chesapeake Room A/B)

Sunday, February 23, 1997

7:45 AM–8:00 AM  President’s Welcome and Opening Remarks from Program and Local Arrangements Chairpersons (Constellation Ballroom A)
8:00 AM–4:30 PM  ASA Meeting (Constellation Ballroom A)
8:00–9:00 AM  Serono Lecture: “Genetics of Prostate Cancer” (Constellation Ballroom A)
9:00 AM–5:00 PM  Exhibits Open (Constellation Ballrooms E, F)
9:00–10:00 AM  American Urological Association Lecture: “New Medical Treatments of Impotence” (Constellation Ballroom A)
10:00–10:30 AM  Refreshment Break/Exhibits (Constellation Ballrooms E, F)
10:30–12:00 NOON  Oral Session I: “Genes and Male Reproduction” (Constellation Ballroom A)
12:00 NOON–1:30 PM  Lunch (on your own)
12:00 NOON–1:30 PM  Business Meeting 12:00 NOON–12:30 PM  Speaker and Lunch 12:30–1:30 PM
1:30–3:00 PM  Symposium I: “Regulation of Testicular Growth and Function” (Constellation Ballroom A)
3:00–3:30 PM  Refreshment Break/Exhibits (Constellation Ballrooms E, F)
3:30–4:30 PM  Oral Session II: “Calcium Channels and Male Reproduction” (Constellation Ballroom A)
4:30 PM–6:30 PM  Poster Session I (Constellation Ballrooms C, D)
7:30 PM–11:00 PM  Evening Social and Buffet Dinner (National Aquarium)
### Monday, February 24, 1997

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00 AM–8:00 AM</td>
<td>Past-Presidents' Breakfast (Pratt/Calvert Rooms)</td>
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<td>8:00 AM–4:30 PM</td>
<td>ASA Meeting (Constellation Ballroom A)</td>
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<tr>
<td>8:00–9:00 AM</td>
<td>Oral Session III: “Sex Accessory Organs” (Constellation Ballroom A)</td>
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<tr>
<td>9:00 AM–5:00 PM</td>
<td>Exhibits Open (Constellation Ballrooms E, F)</td>
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<tr>
<td>9:00–9:30 AM</td>
<td>ASA Lecture: “The Use and Abuse of Testicular Sperm As Viewed From A Thirty Year Experience With Epididymal Reconstructive Surgery” (Constellation Ballroom A)</td>
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<tr>
<td>9:30–10:00 AM</td>
<td>Refreshment Break/Exhibits (Constellation Ballrooms E, F)</td>
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<tr>
<td>10:00–11:00 AM</td>
<td>Pharmacia &amp; Upjohn Lecture: “Sex Chromosome Genes and Spermatogenesis” (Constellation Ballroom A)</td>
</tr>
<tr>
<td>11:00–12:00 NOON</td>
<td>Buckeye State-of-the-Art Lecture: “Reproductive Rescue of Endangered Species” (Constellation Ballroom A)</td>
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<td>12:00–1:30 PM</td>
<td>Lunch (on your own)</td>
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<td>12:00 NOON–1:30 PM</td>
<td>Simultaneous Events:</td>
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<td>Editorial Board Meeting/Luncheon (Executive Boardroom)</td>
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<td></td>
<td>Laboratory Science Forum Luncheon (Maryland Suites-Columbia Room)</td>
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<tr>
<td>1:30–3:00 PM</td>
<td>Symposium II: “Estrogen in the Male: Hormonal Carcinogenesis, Toxicology, and Role in Normal Reproductive Function” (Constellation Ballroom A)</td>
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<tr>
<td>3:00–3:30 PM</td>
<td>Refreshment Break/Exhibits (Constellation Ballrooms E, F)</td>
</tr>
<tr>
<td>3:30 PM–4:30 PM</td>
<td>ASA Business Meeting/Awards Ceremony (Constellation Ballroom A)</td>
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<tr>
<td>4:30 PM–5:00 PM</td>
<td>Exhibitors Prize Drawing (Constellation Ballrooms E, F)</td>
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<tr>
<td>5:00 PM–7:00 PM</td>
<td>Poster Session II (Constellation Ballrooms C, D)</td>
</tr>
<tr>
<td>7:00 PM–8:00 PM</td>
<td>Student Colloquium “On Human Destiny” (Maryland Suites-Annapolis/ Baltimore Rooms)</td>
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### Tuesday, February 25, 1997

<table>
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<th>Time</th>
<th>Event</th>
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<tr>
<td>8:00–12:00 NOON</td>
<td>Andrology Laboratory Workshop (location to be announced)</td>
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<tr>
<td>8:00–9:30 AM</td>
<td>Oral Session IV: “Andrology and Assisted Reproductive Techniques” (Constellation Ballroom A)</td>
</tr>
<tr>
<td>9:30–10:00 AM</td>
<td>Refreshment Break/Exhibits (Constellation Ballrooms E, F)</td>
</tr>
</tbody>
</table>
| 10:00–12:00 NOON | Plenary Session: “Normal and Abnormal Prostate” 
ASA/National Medical Enterprises State-of-the-Art Lecture: “Gene Therapy of Prostate Cancer” (Constellation Ballroom A) |
| 12:00–1:00 PM    | Lunch (on your own)                                                  |
| 1:00–5:00 PM     | Andrology Laboratory Workshop (location to be announced)             |
22nd Annual Meeting

Sunday, February 23

7:45–8:00 AM  Welcome and Opening Remarks (Constellation Ballroom A)
Arnold Belker, President
Terry Brown, Local Arrangements Chairperson

8:00–9:00 AM  Serono Lecture (Constellation Ballroom A)
Patrick Walsh “Genetics of Prostate Cancer”
Chairperson: Dolores Lamb

9:00–10:00 AM  AUA Lecture (Constellation Ballroom A)
Irwin Goldstein “New Medical Treatments of Impotence”
Chairperson: Wayne Hellstrom

10:00–10:30 AM  Refreshment Break/Exhibits (Constellation Ballrooms E, F)

10:30–12:00 noon  Oral Session I
Genes & Male Reproduction (Constellation Ballroom A)
Chairpersons: Shalender Bhasin, Craig Niederberger

10:30 AM  1 cDNA cloning, chromosomal mapping, and tissue-distribution of the human axonemal
dynein light chain gene: A candidate gene for the immotile cilia syndrome / K. Kastury,
W.E. Taylor, S. Arver, C.E. Fisher, M. Gutierrez, S. Bhasin

10:45 AM  2 Y-Deletions in men with severe oligosperma / E.J. Meuleman, J.A. Kremer, J.H. Tuerlings

11:00 AM  3 Hst-7: A male sterility mutation perturbing sperm motility, flagellar assembly, and mitochon­
drial sheath differentiation / S.H. Pilder, P. Olds-Clarke, J.M. Orth

11:15 AM  4 Dysplasia of the fibrous sheath and dynein deficiency, a cytoskeletal abnormality of hu­
man spermatozoa / H.E. Chemes

11:30 AM  5 Interaction of sterility factors in the t haplotype prevents sperm penetration of the zona­
free egg / P. Olds-Clarke, L. H. Johnson, S.H. Pilder

11:45 AM  6 Cloning and characterization of the human SCP1 gene encoding a major component of
meiotic synaptonemal complexes / N. Kondah, Y. Nishina, M. Koga, K. Uchida, H. Tanaka,

12:00–1:30 PM  Lunch (on your own)
12:00–1:30 PM  Women in Andrology Luncheon (Chesapeake Room A/B)
Anna Steinberger “Successful Mentoring”
Chairpersons: Donna Vogel, Dolores Lamb
Business Meeting 12:00–12:30
Speaker and Lunch 12:30–1:30

1:30–3:00 PM  Symposium I: “Regulation of Testicular Growth and Function” (Ballroom A)
Chairpersons: Ken Roberts, Michael Griswold
Patricia Morris “Testicular JAK-STAT Signalling Pathways: Cytokines, Growth Factors,
and Spermatogenesis”
Martin Matzuk “Transgenic Models to Study Male Reproductive Function”

3:00–3:30 PM  Refreshment Break/Exhibits (Constellation Ballrooms E, F)

3:30–4:30 PM  Oral Session II
Calcium Channels and Male Reproduction (Ballroom A)
Chairpersons: Susan Benoff, Joel Marmar

3:30 PM  7 Investigation into the mechanism underlying infertility associated with calcium (Ca2+) channel blockers / A. Jacob, I.R. Hurley, A. Hershlag, S. Benoff

3:45 PM  8 Isolation and characterization of the rat testes-specific voltage dependent calcium (Ca2+) channel (VDCC) / L.O. Goodwin, N.B. Leeds, S. Benoff

4:00 PM  9 A challenge to the concept that the use of calcium channel blockers cause reversible male infertility / D. Katsoff, J.H. Check, D.J. Check

4:30–6:30 PM

POSTER SESSION I—Includes Student Award Candidates
(Constellation Ballrooms C, D)

Sperm


12 Signal transduction by novel sperm chemoreceptors as a mechanism for mammalian sperm-egg chemotaxis / L.D. Walensky, M. Ruat, R. Bakin, G.V. Ronnett, S.H. Snyder

13 Calcium flux through sperm inositol 1,4,5-trisphosphate receptors is implicated in triggering the mammalian acrosome reaction / L.D. Walensky, S.H. Snyder


15 Effect of tumor necrosis factor (TNF-α) and interferon (IFN-γ) on human sperm motility, viability, and motion parameters / H.C. Champion, L.S. Estrada, R. Wang, A. Baratta, M. Rajaskaran, S.C. Sikka, W.J.G. Hellstrom

16 Antioxidant capacity of seminal plasma compared to synthetic and biological free radical scavengers / J.S. Armstrong, M. Rajaskaran, A. Baratta, W.J.G. Hellstrom, S.C. Sikka


19 Transesterification of human sperm phospholipids with exogenous fatty acid coenzyme A thioesters / J.G. Alvarez, S. Balgobin

20 A role for SGP-1 in binding of sperm to eggs / R.H. Hammerstedt, P. Cramer, G.F. Barbato, R.P. Amann

Epididymis

21 Epididymal protein pattern in infertile ornidazole treated rats / A. Wagenfeld, C.H. Yeung, T.G. Cooper

22 Immunoexpression of FAS (CD95) ligand (FasL) in the rat testis and epididymis / F.C. Griffin, D.F. Cameron


25 The status of cauda epididymal sperm DNA following partial sympathetic denervation / M. Schwartz, D.D. Ricker

26 Epithelial cell pathology of the epididymis in adult and elderly men / J. Regadera, F. Martinez-Garcia, P. Cobo, J. Palacios, M. Nistal

Environment and Reproduction

27 Urinary creatine as a biomarker for lead induced testicular damage / S.R. Skaggs, W.J. Moorman, D.D. Sharpnack, S.M. Schrader


29 Sertoli cells in culture and mRNA differential display provide a sensitive early warning assay system to detect xenobiotics / V. Syed, W. Gu, N.B. Hecht

30 A morphological study of the epididymis and testis of rats UChA and UChB submitted to experimental chronic alcoholism / E. Bustos-Obregon, F.E. Martinez, M. Martinez, V.H.A. Cagnon, P.J. Garcia, C.R. Padovani, H.R. Contreras

31 No decline in Sertoli cell number or function in men over the last 16 years / L. Johnson, J.J. Barnard

32 No decline in spermatogenic potential in a group of North American men over the last 16 years / L. Johnson, J.J. Barnard, H. Meguerditchian

33 Decline in Leydig cell volume in a group of North American men over the last 16 years / L. Johnson, S.W. Brown, J.J. Barnard
Impotence


35 Age-related alterations in plasma nitrite and nitrate levels in impotent men / M. Rajasekaran, W.J. Hellstrom, S.C. Sikka


37 \( \alpha \)-Blockers enhance penile erection effect of PGE\(_1\), in cats / R. Wang, H. Champion, S.C. Sikka, P. Doherty, W.J.G. Hellstrom


40 Androgen regulation of rat penile erection / C.M. Reilly, V.S. Stopper, T.M. Mills

Prostate

41 Assessment of the male accessory glands of infertile men with kallikrein hK2 in seminal plasma / R.R. Tremblay, E. Coulombe, G. Frenette, J.Y. Dubé

42 The effects of spinal cord injury on TRPM and androgen receptor mRNA in the prostate of the rat / H.F.S. Huang, M-T Li, T.A. Linsenmeyer, J.E. Ottenweller, L.M. Pogach, R.J. Irwin

43 Isolation and purification of basal and secretory epithelial cells from the rat ventral prostate / N. Ravindranath, M. Dym

44 Intraprostatic injection of zinc salt as a treatment for benign prostatic hyperplasia / M. Wang, J. Cao, M.S. Fahim

45 Osteopenia in men with prostate cancer treated with androgen deprivation / D.A. Cook, M. Eisenberger, C.R. Schneyer, D. Hoover, A.S. Dobs

46 The regulation of prolactin and EGF receptor mRNAs in human prostate cancer cell line (LNCaP) by LHRH / J-Y, Liang, J.N. Rao, J.F. Wilber, P. Feng


48 The cynomolgus monkey prostate under physiological and hypogonadal conditions: an ultrasonographic study / A. Kamischke, H.M. Behre, G.F. Weinbauer, E. Nieschlag

Testis

49 Developmental shift of inhibin or inhibin related proteins producing site in fetal through infantile rat testes / J. Noguchi, H. Hikono, S. Sato, K. Kikuchi, A. Shimada, H. Kaneko, Y. Hasegawa, G. Watanabe, K. Taya

50 Local testicular control mechanism enabled the total number of Sertoli cells found in an intact rat to be exceeded / L. Johnson, K.L. Kunde, G.E. Keilior, A.J. Kaschmitter


52 Paternal age affects progeny outcome in the Brown Norway Rat / V. Serre, B. Robaire

53 Stage-specific loss of germ cells in the rat after a single exposure to heat occurs by apoptosis / Y.H. Lue, A.P.S. Hikim, P. Im, K.S. Tiang, T. Bui, A. Leung, R. Swerdloff, C. Wang

54 Biochemical characterization of Sertoli cell (SC) membrane proteins that are likely to be involved in specialized junctional complex (JC) formation / D Mruk, W.M. Lee, C.Y. Cheng

55 TPX-1 gene is specifically transcribed by normal human germ cells during the late stage of spermatogenesis / N. Tokuda, S. Matsuzaki, K. Nomoto, J. Kumazawa

56 Sertoli cell cytoskeletal and related proteins, and \( \alpha \)-macroglobulin, are not involved in inhibited spermiogenesis (I.S.) / R.N. Wine, R.E. Chapin

57 5-Azacytidine treatment initiated during meiotic development of male germ cells results in abnormal embryo development / T.E. Doerksen, J.M. Trasler

58 Expression of cyclin H and cyclin-dependent kinase 7 in the mouse testis / J.T. McGaughy, S.E. Ravnik

59 Effect of thyroid hormone on testicular development and mRNA levels of thyroid hormone receptors in the testes of immature and adult rats / J.N. Rao, J-Y. Liang, J.F. Wilber, P. Feng
Identification of two kinetically distinct activities of 11β-hydroxysteroid dehydrogenase in rat Leydig cells / R.S. Ge, H.B. Gao, V. Lakshmi, G.L. Gunsalus, M.P. Hardy

Monday, February 24

7:00–8:00 AM  Past President’s Breakfast (Pratt/Calvert Rooms)

8:00–9:00 AM  Oral Session III
   Sex Accessory Organs (Constellation Ballroom A)
   Chairpersons: Barry Hinton, Donald Tindall

8:00 AM  61 Nitric oxide as a factor in growth of the canine prostate / J.K. Crone, S.L. Chamness, T.S.K. Chang, J.D. Strandberg, A.L. Burnett
8:30 AM  63 Loss of testicular factors causes changes in the stability and transcription rate of gamma-glutamyl transpeptidase mRNA-IV in the initial segment of the rat epididymis / D.B. Rudolph, B.T. Hinton
8:45 AM  64 Ductuli efferentes in estrogen receptor knock-out mice do not reabsorb luminal fluid / R.A. Hess, D. Bunick, J. Bahr, D.B. Lubahn

9:00–9:30 AM  ASA Lecture (Constellation Ballroom A)
   Robert Schoysman “The Use and Abuse of Testicular Sperm As Viewed From A Thirty Year Experience With Epididymal Reconstructive Surgery”
   Chairperson: Arnold Belker

9:30–10:00 AM  Refreshment Break/Exhibits (Constellation Ballrooms E, F)

10:00–11:00 AM  Pharmacia & Upjohn Lecture (Constellation Ballroom A)
   Colin Bishop “Sex Chromosome Genes and Spermatogenesis”
   Chairperson: Peter Schlegel

11:00–12:00 NOON  Buckeye State-of-the-Art Lecture (Constellation Ballroom A)
   JoGayle Howard “Reproductive Rescue of Endangered Species”
   Chairperson: Terry Brown

12:00–1:30 PM  Lunch (on your own)

12:00–1:30 PM  Simultaneous Events
   1. Laboratory Science Forum (Maryland Suites-Baltimore Room)
      Grace Centola “The Basics of Gamete Cryopreservation”
      Chairperson: Grace Centola
   2. Editorial Board Meeting/Luncheon (Executive Boardroom)

1:30–3:00 PM  Symposium II (Constellation Ballroom A)
   “Estrogen in the Male: Hormonal Carcinogenesis, Toxicology, and Role in Normal Reproductive Function”
   Chairperson: Rebecca Sokol
   John McLachlan “Hormonal Carcinogenesis and the Male”
   Stephen Safe “Environmental Estrogens and the Male”

3:00–3:30 PM  Refreshment Break/Exhibits (Constellation Ballrooms E, F)

3:30–4:30 PM  ASA Business Meeting and Awards Ceremony (Constellation Ballroom A)
POSTER SESSION II (Constellation Ballrooms C, D)

Animal Science

65 Effect of testicular biopsy and triethylenemelamine on testis function and semen quality in the dog / R.H. Foote
66 Sensitivity of domestic cat sperm to cold-induced acrosomal damage / B.S. Pukazhenthi, K.M. Pell, D.E. Wildt, J.G. Howard
68 Ejaculate characteristics of the black-handed spider, southern black howler and diana monkey / J.A. Long, N. Lamberski, A.H. Shoemaker
69 Repeated follicular stimulation in the endangered lion-tailed macaque (Macaca silenus) with recombinant human gonadotrophins / M. Cranfield, N. Berger, J. Liu, J. Smart, D. Summers
70 The use of intracytoplasmic sperm injection (ICSI) to enhance the conservation of the endangered lion-tailed macaque (LTM) (Macaca silenus) / M. Cranfield, J. Liu, D. Summers, J. Smart, N. Berger
71 Increased fertility in turkeys resulting from semen donor selection / A.M. Donoghue
73 Sperm-binding assay to detect males likely to have relatively low fertility / R.H. Hammerstedt, S.P. Gill, R.P. Amann, PC. Cramer, G.F. Barbato
74 Testicular morphology and gonadotropin receptors reflect age-related alterations in function / M.A. Ottenger, N. Thompson, M.R. Bakst, K. Kubakawa, K. Kubakawa, M. Kikuchi, S. Ishii
75 Variability and power calculations of viability estimates in dutch belted rabbit sperm / M.J. Breitenstein, S.M. Schrader, S.D. Simon
76 Role of inhibin in the regulation of FSH secretion in immature bulls / H. Kaneko, J. Noguchi, K. Kikuchi, A. Shimada, G. Watanabe, K. Taya, Y. Hasegawa, K. Okuda

Sperm Processing

77 Comparison of monopercoll sperm separation with two-layer percoll method / K. Seifarth, D. Garlak, L. Cordek, C. Fitzhugh, A.J. Thomas, Jr., A. Agarwal
78 Microscopic sperm preparation might be a novel approach for intracytoplasmic sperm injection (ICSI) / A. Hossain, S. Barik, B. Rizk, S. Lynn Jr., I. Thorneycroft
81 Processing method of frozen donor sperm correlates with final sperm parameters and intrauterine insemination pregnancy outcome / S.K. Jindal, K.J. Lipetz
82 Frozen human sperm maintains motility following processing and refreezing / P.J. Jones, L. Thanh, J. Schulman, E. Fugger
83 Post-thaw plasma membrane integrity (PMI) of mouse sperm cryopreserved in media containing polyols and/or trehalose or raffinose / K.A. Thompson, E.E. Noiles, B.T. Storey
84 Treatment of human or bull sperm with a synthetic peptide increases binding to egg membrane substrate / R.P. Amann, R.H. Hammerstedt, R.B. Shabanowitz
85 Gender preselection in mammals: An update on flow cytometric X/Y sperm sorting / L.A. Johnson, G.R. Welch, W. Rens

Clinical Andrology

86 Vasectomy, vasovasostomy and MESA / ICSI: Is it the future triad of vasectomised man who regrets vasectomy? / M.T.W.T. Lock, H. Roshani
87 Evaluation of men with varicoceles for IVF, ICSI or other options / D. Glazier, J.L. Marmar, M. Gibbs, S.L. Corson
88 Micronuclei and other nuclear abnormalities in buccal mucosa in people exposed to testosterone or gonadotrophines / M. Diaz, O. Torres-Bugarin, E. Vargas, M.F. Ramierz-Munoz, J. Sanchez-Corona, G. Zuniga
90 Testicular microlithiasis (TML) in men with azoospermia and severe oligospermia / I. H. Hirsch, R.I. Feld, L.G. Gomella, PT. McCue
91 Are sperm motion parameters influenced by varicocele ligation? / I.H. Hirsch, M.T. Ismail, J. Sedor
93 Intruterine insemination for cervical or male factor problems in women >40 have very poor viable pregnancy rates / J.H. Check, D. Lurie, M. Peymer, D. Katsoff, R. Long
94 Absence of glucose decreases human fertilization and sperm movement characteristics in vitro / M.M. Mahadevan, M.M. Miller, D.M. Moutos
95 Improvement in semen quality and frequency of spontaneous acrosome reaction after pentoxifylline addition in normozoospermic men / S.C. Esteves, R.K. Sharma, A.J. Thomas, A. Agarwal
96 Effect of peritoneal fluid on sperm motion characteristics and acrosome reaction in women with endometriosis / Y. Wang, R.K. Sharma, T. Falcone, A. Agarwal
98 Vasectomy-related infertility: A common and expensive medical problem / A.M. Jequier
99 Progressive motility in computer-assisted sperm analysis (CASA) / V.L. Slott, S.D. Perreault
100 Conditioned media from cumulus cell cultures stimulate in vitro acrosome reaction / G.M. Centola, S.P. Weisensel, V.L. Lewis
101 Mitochondrial DNA, semen quality and clinical assessment / A.M. Jequier, R. Martin, D. Mehmet, J. Goldblatt, J.M. Cummins
103 A new category of infertility due to sperm-specific abnormality of CD46 / M. Kitamura, K. Matsumiya, M. Yamanaka, A. Okuyama

Contraception

105 Vaginal delivery of new formulations of nonoxynol-9 co-precipitated with polyvinylpyrrolidone: assessment of two formulation-delivery systems on the onset of pregnancy in rabbits / P.M. Zavos, J.R. Correa
106 Assessment of spermicidal efficacy of new formulations of nonoxynol-9 coprecipitated with polyvinylpyrrolidone in rabbits: comparison between two formulation-delivery systems / P.M. Zavos, J.R. Correa
107 Effects of an inhibitor of adenylate cyclase (AC) on sperm proacrosin activation / T. Toda, Y. Yamamoto, I. Miyagawa, P.M. Zavos, N. Sofikitis
108 Cyproterone acetate (CPA) plus testosterone enanthate (TE) 100 mg/week induces a dose-dependent suppression of spermatogenesis in normal men / M.C. Meriggiola, C. Flamigni, W.J. Bremner

Male Infertility Diagnosis

110 Test kit for the determination of sperm viability / J.G. Alvarez, S. Balgobin, R.D. Powers, G. Davis
111 Test kit for the determination of leukocyte count in semen / J.G. Alvarez, S. Balgobin, R.D. Powers, G. Davis
112 The sperm penetration assay: an important tool in the age of ICSI / J.D. Wininger
113 The fertility score test kit® detects high sperm density but is not as sensitive for low density / E.V. Younglai, G. Tuke, J.A. Collins, A. Vawda
114 Correlation between mannose ligand receptor expression, acrosome reaction, Kruger’s morphology and sperm motility in normozoospermic men / R.K. Sharma, A.J. Thomas Jr., A. Agarwal
115 Role of electron microscopy in the evaluation of male infertility during the era of ICSI / D. Carbone, J. McMahon, H. Levin, A.J. Thomas Jr., A. Agarwal
116 Sperm function tests in the assessment of semen quality / R.S. Sidhu, R.K. Sharma, Y. Wang, A.J. Thomas Jr., A. Agarwal
117 Differences in the manual and CASA results in semen specimens before and after freezing / R.S. Sidhu, R.K. Sharma, A.J. Thomas, A. Agarwal

118 Lipid peroxidation in cryopreserved semen from cancer patients / Y. Wang, R.K. Sharma, A.J. Thomas, A. Agarwal


120 Correlation of hypoosmotic swelling (HOS) test with vitality staining, motility and spermatic morphology / A. Arredondo-Pineda, C. Bravo-Gatica, P. Troncoso-Torrez, R. Tapia-Serrano

7:00–8:00 PM Student Colloquium (Maryland Suites-Annapolis/Baltimore Rooms)
“On Human Destiny” Donald Coffey
Chairperson: Grace Centola

Tuesday, February 25

8:00–9:30 AM Simultaneous Event
Oral Session IV (Ballroom A)
Andrology and Assisted Reproduction Techniques
Chairpersons: Luis-Rodriguez-Rigau, Juan Alvarez

8:00 AM 121 Different techniques to identify and label human round spermatids / L.T. Colombero, P.N. Schlegel, M. Feliciano, M.L. Papale, M. Goldstein, Z. Rosenwaks, G.D. Palermo

8:15 AM 122 Cryopreservation of sperm collected by TESE provides adequate post-thaw viability and successful IVF-ICSI pregnancies / R. Dolgina, G. Wolf, P. Studney, B. Kaplan, C. Niederberger, L. Ross, G.S. Prins

8:30 AM 123 Successful intercontinental genome resource banking and artificial insemination with cryopreserved sperm in cheetahs. J.G. Howard, T.L. Roth, W.F. Swanson, J.L. Buff, M. Bush, J. Grisham, L. Marker-Kraus, D. Kraus, D.E. Wildt

8:45 AM 124 Interpretation of CASA data using distribution based and multivariate statistical methods / D. Higdon, M. Lavine, J. Liu, S. Perreault, V. Slott, D. Katz


9:15 AM 126 Lower concentration of sperm used for oocyte insemination for in vitro fertilization (IVF) may result in higher pregnancy rates: A preliminary study / J.H. Check, C. Hourani, K. Benfer, A. Baker

9:30–10:00 AM Refreshment Break/Exhibits (Constellation Ballrooms E, F)

10:00–12:00 NOON Plenary Session “Normal and Abnormal Prostate” (Ballroom A)
Chairpersons: Gail Prins, Glenn Cunningham
ASA/National Medical Enterprises (NME) State-of-the-Art Lecture “Gene Therapy of Prostate Cancer” Jonathan Simon

“Novel Markers of Benign and Malignant Prostatic Disease—Do They Help in Diagnosis?” William Nelson

“Androgen Regulated Prostatic Gene Expression” Martin Tenniswood

8:00–12:00 NOON Simultaneous Event
Andrology Laboratory Workshop (location to be announced)
“Moving Beyond Boundaries: Clinical Andrology in the 21st Century”
Chairperson: Christopher De Jonge
Program Directors: Christopher Barratt, Pasquale Patrizio

8:00–9:15 AM “Introduction to Molecular Biology I: The Language” Ines Moretti-Rojas

9:15–10:10 AM “Introduction to Molecular Biology II: The Methods” Ines Moretti-Rojas

10:10–10:30 AM Break
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<td>10:30-11:15 AM</td>
<td>&quot;Recombinant Protein Technology in the Andrology Lab&quot; Christopher Barratt</td>
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<tr>
<td>11:15-12:00 AM</td>
<td>&quot;The Use of Molecular Biology to Define Molecules Involved in Gamete Recognition and Signal Transduction During Fertilization&quot; Greg Kopf</td>
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<td>12:00 NOON</td>
<td>Lunch (on your own)</td>
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<td>1:30-2:30 PM</td>
<td>&quot;Clinical Application of Molecular Biology: Cystic Fibrosis Mutations, Y-Chromosome Deletions and the Future&quot; Mary Jo Kent-First, Pasquale Patrizio</td>
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<td>2:30-3:15 PM</td>
<td>&quot;DNA Breakage: Why is it Important for the Andrologists?&quot; Donald Evenson</td>
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<td>3:15-3:30 PM</td>
<td>Break</td>
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<td>3:30-4:00 PM</td>
<td>Break Out Session—Group Problem Solving</td>
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<td>1. How to set up a service screening for Y-chromosome deletions for men attending ART clinics.</td>
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<td>2. How to set up a testis library and what is the most effective way to screen the library</td>
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<td>3. A purified protein of interest is supplied to your lab. How do you go about cloning the gene.</td>
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<td>4:00-4:45 PM</td>
<td>Group Discussion of Problems</td>
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<td>4:45-5:00 PM</td>
<td>Concluding Remarks</td>
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and with no other causal factors. We recommend DNA screening for microdeletions in the AZFc region of the Y chromosome to be performed in men with severe oligospermia. The nature of the diagnostic procedure (PCR analysis) and the method of DNA screening in DNA samples from men with these defects are described in detail. 

In this study we investigated the prevalence of microdeletions in the AZF region of the Y chromosome in our ICSI population by PCR analysis and looked for clinical differences between the men with and without the deletion. Blood was drawn from 154 men, who were waiting for ICSI treatment: 24 azoospermic men, 98 oligospermic and 32 normospermic men. (previous fertilization failure). Chromosome analysis showed 4 Klinefelters in the azoospermic group and two Klinefelters in the oligospermic group. One translocation was observed in the oligospermic group. Microdeletions in the AZF region were present in 7 of the 98 oligospermic men (7%). None of these 7 men had abnormal findings on andrologic history and examination. No microdeletions were found in the azoospermic and normospermic group.

We conclude that microdeletions in the AZF region of the Y chromosome are frequently found in men with severe oligospermia and with no other causal factors. We recommend DNA screening (and genetic counseling) in this population of subfertile men.

2. Y-DELETIONS IN MEN WITH SEVERE OLIGOSPERMIA.

E.J. Meuleman, J.A. Kremer, J.H. Tuerlings, Departments of Urology, Obstetrics & Gynecology and Human Genetics, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

The introduction of ICSI offered a successful treatment option for subfertile male with severe oligospermia, although the etiology of the disorder remains unclear in most cases. Recently, microdeletions in the AZF region of the Y chromosome were detected in men with azoospermia or severe oligospermia. In this study we investigated the prevalence of microdeletions in the AZF region of the Y chromosome in our ICSI population (by PCR analysis) and looked for clinical differences between the men with and without the deletion. Blood was drawn from 154 men, who were waiting for ICSI treatment: 24 azoospermic men, 98 oligospermic and 32 normospermic men. (previous fertilization failure). Chromosome analysis showed 4 Klinefelters in the azoospermic group and two Klinefelters in the oligospermic group. One translocation was observed in the oligospermic group. Microdeletions in the AZF region were present in 7 of the 98 oligospermic men (7%). None of these 7 men had abnormal findings on andrologic history and examination. No microdeletions were found in the azoospermic and normospermic group.

We conclude that microdeletions in the AZF region of the Y chromosome are frequently found in men with severe oligospermia and with no other causal factors. We recommend DNA screening (and genetic counseling) in this population of subfertile men.

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**His-7:** A MALE STERILITY MUTATION PERTURBING SPERM MOTILITY, FLAGELLAR ASSEMBLY, AND MITOCHONDRIAL SHEATH DIFFERENTIATION

S.H. Pilder, P. Olds-Clarke, and J.M. Orth, Department of Anatomy & Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140.

His-7 is a novel mouse mutation, mapping to the haplotype region of chromosome 17, a large portion of the chromosome known to include several genetic factors which cause defects in sperm function and/or differentiation. Male laboratory mice heterozygous for the wildtype (+) allele and an allele derived from another mouse species, M. spreus (s), are sterile as the result of poor sperm motility. Slight but significantly less s/+ sperm are motile relative to +/+ control sperm, and the mutant sperm exhibit a net velocity of about half that of +/+ sperm. Male mice heterozygous for the s allele and a haplotype allele (o) have only about half the number of motile sperm as the +/+ control animals, and these male sperm exhibit so little forward movement that it is below the detectable limit of the sperm motility analysis system.

Light microscope observations of s/+ cauda epididymal sperm reveal that most appear normal or near normal. However, similar observations of s/o sperm reveal a number of abnormalities. For example, sperm including midpiece bubbles, shortened tails, normal heads attached to multi-dentated "flagella" (reminiscent of the previously reported His-5 homologous allele, Piller et al., 1993, Devel. Biol. 159, 631-642), and many disorganized sperm heads and tails.

Because of these observations, more detailed studies of sperm ultrastructure were undertaken. Abnormalities include: abnormalities of the sperm midpiece and axonemal anomalies like missing dynein arms and these defects are made less apparent by the ameliorative affects of the + allele. In any event, His-7 offers us the opportunity to study asthenospermia and spermatic differentiation at the molecular level.

Research supported by NIH grant HD31164 and NSF grant 9513464.

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**DISPLASIA OF THE FIBROUS SHEATH AND DYEINE DEFICIENCY. A CYTOSKELETAL ABNORMALITY OF HUMAN SPERMATOZOA.**

H.E. Cheek, Laboratory of Testicular Pathology, Buenos Aires Children's Hospital, Argentina. In 1987 we described the dysplasia of the fibrous sheath as a serious defect of human spermatozoa in five sterile patients with severe asthenozoospermia. We have gathered a large series of 34 patients and defined the clinical and seminal features of this syndrome. The patients are young males in their third and forth decades of age, consulting for primary sterility. Seven of them have chronic sinusitis, bronchitis, and or bronchiectasis from early childhood. Two of the patients were brothers (not twins). Seminal features include rigid, short and thick flagella, extreme asthenozoospermia, and various degrees of necrozoospermia. On ultrastructural examination the characteristic is a serious distortion of the fibrous sheath (FS) with hyper trophy and hyperplasia of its constitutive elements, frequent deficiencies of microtubular assembly at the middle piece and axonemal anomalies like missing dynein arms and lack of central doublets (9+0 axonemes). The patients can be divided in two subgroups defined by the prevalence of the fibrous sheath anomalies in their spermatozoa.

The complete form (n=21) shows 0,71,4% total motility (TM), with 0,4% fast forward motility (FFM). All spermatozoa have severe FS alterations. In the incomplete form (n=13) TM was 7,9±7,3% and FFM 0,6±1,4%. Tail alterations were similar, but there were 20-30% of spermatozoa with normal fibrous sheaths. Dead spermatozoa ranged between 4 and 75%, with 11 patients showing values higher than 40%. There were no spontaneous or IVF pregnancies. ICSI has been recently performed with success in 2 cases. Lack of dynein arms was found in bronchial cilia in the two patients with respiratory symptoms in which a biopsy was performed. They constitute a variant of the immotile cilia syndrome. Regardless of various treatments, seminal and clinical characteristics remained stable in all the patients which were followed for different periods of time. The features described configure a phenotype that suggests a genetic origin of the syndrome.
5 INTERACTION OF STERILITY FACTORS IN THE r HAPLOTYPE PREVENTS SPERM PENETRATION OF THE ZONA-FREE EGG
P. Ords-Clarke, L.H. Johnson and S.H. Pledger, Department of Anatomy & Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140.

The r haplotype includes several genetic factors which cause defects in sperm function in fertilization and which are linked together within four large inverted regions on mouse chromosome 17. Sperm from mice carrying two complete r haplotypes are unable to penetrate the zona-free oocyte, but the number and location of the gene(s) within the r haplotype which cause the egg penetration defect are not known.

Several sterility factors have been mapped within the r haplotype. S2 is located in the most distal inversion and causes sterility when homozygous. S1 is located in the most proximal inversion; while S1 homozygotes are fertile, males homozygous for S1 and heterozygous for S2 are sterile (Lyon, 1986, Cell 44:3357).

We have shown that sperm from mice homozygous for S2 and heterozygous for S1 were unable to penetrate any eggs even at a high sperm-egg ratio (Johnson et al., Gen. Res. 1995. 66:189), suggesting that the egg penetration defect was located in S2. However, when sperm from mice homozygous for S2 were tested at two different sperm-egg ratios (1:00:1 and 100:1), and compared to fertile controls in the same experiment, the mutant sperm penetrated 10.6±13% of the mean percent of eggs penetrated by wildtype sperm at the higher sperm-egg ratio, and 13.6±5% at the lower sperm-egg ratio (N=3).

This indicates that interaction of gene(s) in S1 with S2 is an important component of the r haplotype's effect on egg penetration. This interaction could be via a signal transduction pathway necessary for egg penetration.

Research supported by NSF grant No. 9513464.

6 CLONING AND CHARACTERIZATION OF THE HUMAN SCP1 GENE ENCODING A MAJOR COMPONENT OF MEIOTIC SYNAPTICAL COMPLEXES
N. Kondoh1, Y. Nishina2, M. Koga1, U. Ichida1, H. Tanaka1, M. Kitamura1, K. Matsumiya1, Y. Nishimune1 and A. Okuyama1. Department of Urology, Osaka University Medical School, Osaka Institute for Microbial Diseases, Osaka University, Osaka, 565, Japan.

We have identified several differentiation-specific molecules of germ cells using germ cell-specific antibodies and among them, human synaptosomal complex protein 1 (SCP1) was cloned from human testis cdNA libraries. The 3.5 kb gene encodes a major component of transverse filaments of synaptonemal complexes (SCs). SCs are meiosis-specific structures playing a key role in chromosome paring and recombination at the meiosis prophase. The human SCP1 cdNA contained the complete coding region of 973 amino acids, which included several important motifs, such as the coiled-coil region, leucine zipper and many target sites of several kinases including p38 and protein kinase. Northern blot analysis showed testis-specific expression of SCP1 and the gene was localized onto human chromosome 1q13 by fluorescence in situ hybridization. Further analysis of human SCP1 gene-related molecules could be a clue of elucidation of spermatogenic arrest causing male infertility.

7 INVESTIGATION INTO THE MECHANISM UNDERLYING INFERTILITY ASSOCIATED WITH CALCIUM (Ca++) CHANNEL BLOCKERS A. Eshri, R. A. Shrie, A. Hershlag and S. Benoff, Division of Human Reproduction, North Shore University Hospital, Manhasset, NY.

Clinical dosages of lipophilic Ca++ entry antagonists (Ca++ channel blockers: nifedipine, diltiazem) which act as the prototype dihydropyridine-type calcium channel blockers, is identified as a major cause of male infertility in men with normospermic semen (Fertil. Steril. 1994 62:606-617). These drugs suppress the surface expression of mannoside receptors (MR, zona recognition factor) causing the free cleavage plane of the zona pellucida (ZP). However, based on drug design, it is hypothesized that CCBs must interact with voltage-dependent Ca++ channels in the human sperm plasma membrane to produce the infertility state. To test this hypothesis, we have examined the effects of CCBs and exogenous cholesterol on norepinephrine progestosterone receptor (PR) expression and function by female donor sperm. Exposures of sperm to progestosterone (P) induces Ca++ influx through voltage-operated Ca++ channels followed by an acrosome reaction (AR). Prior studies have demonstrated that unlike MR, PR is a constitutively expressed integral sperm plasma membrane protein (ARJU 1993:54:100-113). Fertile donor sperm labelled with fluorescent conjugated progestosterone carboxymethylzyme BSA (P-CMO:BSA) exhibit a time dependent increase in the percentage of sperm expressing PR on their surface membranes during 48hr incubation at 37°C. As determined by gas chromatography, incubation in presence of cholesterol saturated BSA (C-BSA) 34hrs at time zero or after 48hrs in capacitating media causes the sperm plasma membrane cholesterol content to decrease down to that previously observed in infertile men taking CCBs. Such cholesterol loading does not affect PR expression. Consistent with this finding, exposure to sulfated nifedipine did not affect PR expression either at 24hr or 48hr incubation. Nevertheless, the effect of C-B and nifedipine on P-stimulated AR differ significantly. Levels of P-stimulated AR detected using rhodamine labelled Phospha similar antibody or similar antibodies are similar in control and C-BSA treated sperms. While compared to control, 24hr treatment with nifedipine has no effect on P-stimulated AR. However, at 48hr, the response of nifedipine treated sperm to P is markedly reduced. The prolonged unliganded P leads to the presence of nifedipine-blocked P-stimulated AR is well correlated with preliminary findings on the structure of the voltage-dependent Ca++ channel. While an altered dihydropyridine binding site has been identified (ARJU 1996, O-003), in contrast, the blockade of the dihydropyridine binding site has no effect on ionomycin-stimulated AR. This demonstrates that the effect of nifedipine is specific to the voltage-dependent Ca++ channel. These observations indicate that changes in the cholesterol balance affect Ca++ influx through P-stimulated AR. These data demonstrate that the pharmacological activity of CCBs contribute to the production of infertility state. In summary, CCBs interfere with multiple processes regulating sperm fertilizing potential. (Supported by NIH Grant No. ES05100 S.B.)

A case of failed fertilization associated with decreased mannose-ligand receptor expression was reported. The lack of expression of head-directed mannose-ligand receptors at high frequency and the failure to undergo spontaneous acrosome loss was attributed to the use of calcium channel blockers since switching the male partner to an angiotensin-converting inhibitor completely reversed these sperm abnormalities to that of fertile controls. Similarly, 9 additional males taking calcium channel blockers had abnormalities in mannose-ligand binding receptor expression, and in 3 who changed to alternative antihypertensive therapy, there were also changes in semen parameters similar to fertile controls. However, some doubt of the necessity of changing anti-hypertensive drugs was presented at last year's ASA meeting with the demonstration of normal fertilization of oocytes retrieved through IVP and subsequent normal pregnancy rates (PRs) despite the use of calcium channel blockers. The possibility would still exist that the much lower concentration of sperm to which the oocyte is exposed in vivo could still be adversely influenced by the males' use of calcium channel blockers. The study presented herein retrospectively evaluated PRs in patients taking calcium channel blockers attempting conception but not using assisted reproductive technology. There were 11 males identified as taking calcium channel blockers. Pregnancy occurred in 5/11 female partners. The median and mean ages were 33 and 33.8±4.15 for those achieving pregnancy vs 35 and 36.3±5.68 for non-conceivers. Oligosperma and/or asthenosperma was present in 5/6 males not achieving pregnancy. Other contributing factors included tubal factor (n=1), endometriosis (n=1), advanced maternal age (n=2) and ovaulation abnormalities (n=1). These data demonstrate that even in vivo pregnancies are possible despite the use of calcium channel blockers by male partners. Whether the PR is reduced or not would have to be answered by a much larger prospective study.

IDENTIFICATION OF FUNCTIONAL PROGESTERONE RECEPTORS ON THE SURFACE OF HUMAN SPERMATOZOA.

G. Forin, M. Maggi*, E. Baldi*, M. Luconi*, L. Bonaccorsi*, Andrology Unit, Dept. of Clinical Pathophysiology, University of Florence, Italy.

Although the presence of putative progesterone "receptors" on the surface of human sperm has been hypothesized, their precise characterization is still lacking. We investigated the presence of specific progesterone binding sites on capacitated human sperm by using [3H]11-glucuronide-progesterone as a pharmacological tool. Highly motile spermatozoa were separated from human semen by Percoll gradient and incubated with [3H]11-glucuronide-progesterone at 4°C for various times and in the presence of increasing concentrations of progesterone. We found that binding of [3H]11-glucuronide-progesterone to human sperm was time-dependent with a maximum at 1 hour incubation and localized on sperm surface, being almost completely liberated after acidic washing. Simultaneous computer analysis of displacement curves with increasing concentrations of unlabeled progesterone (0.03 nM-100 µM) indicated the presence of two binding sites, respectively with affinity of 0.5 nM (high affinity site) and 32 µM (low affinity site). The other competitors used in our study (17α-OH-progesterone, 11β-OH-progesterone, dihydrotestosterone, testosterone, and estradiol) did not bind to the high affinity site and showed a lower affinity data progesterone for the low affinity site. To determine correlation between binding specificity and biological activity, we studied the effect of progesterone, 17α-OH-progesterone, and 11β-OH-progesterone on [Ca2+]i increase in fura-2-loaded human sperm by a fluorimetric method. Dose-response curves of progesterone were biphasic, showing a first plateau phase between 0.1 and 1 µM, and a second phase in the high micromolar range. Conversely the other sex steroids tested increased sperm [Ca2+]i only in micromolar concentrations. These data indicate a substantial correlation between the biological effect and the binding activity of sex steroids. In conclusion, our study demonstrates the presence of specific, high affinity progesterone binding sites on the surface of human sperm which may be involved in the biological activity of this steroid.

IMPACT OF ABNORMAL SEMEN PARAMETERS ON THE GENETIC STATUS OF HUMAN SPERMATOZOA.


Introduction: Although the use of ICSI has allowed the successful treatment of men with severely abnormal semen, concern exists that this may result in a higher incidence of chromosomal abnormalities as an associated risk. It was the aim of this study to uncover possible relationships between particular semen characteristics and sperm chromosomal abnormalities in normal men.

Material and Methods: Sperm samples from 18 men were processed by density gradient centrifugation, and the sperm characteristics were evaluated according to the WHO and Kruger criteria. Then spermatozoa were smeared on precleaned slides, fixed and analyzed by multiplex FISH for chromosomes 18, X and Y, with particular emphasis on both autosomal and gonosomal aneuploidy and diploidy. Statistical analysis was performed by Spearman correlation coefficient and Pearson x2 test.

Results: A mean of 1.5±0.2 spermatozoa were counted per sample. In 14 men with abnormal semen parameters in regard to concentration, motility and morphology, there was a 2.4% incidence of total chromosomal abnormalities vs. 0.6% in spermatozoa of fertile men (n=4) (P<0.001). Among oligozoospermic men (<20x10^6/ml), the level of such abnormalities was 3.2% vs. 1.3% in normal men (P<0.001). Although there was no overall relationship to sperm motility, the incidence of chromosomal abnormalities was correlated with abnormal morphology (P<0.001), and this was exaggerated further where the incidence of normal forms was ≤50% (P<0.001) compared to those with ≥50% (P<0.01). Finally, when the total number of normal motile spermatozoa fell below 500,000 (the threshold indicator level for ICSI), the incidence of chromosomal abnormalities was 2.6% vs. 1.5% in samples above that threshold (P<0.001). In subfertile men, the most frequent abnormality was diploidy (P<0.001), this being most evident where morphology was ≤50% (P<0.01).

Conclusions: Infertile men have a higher incidence of sperm chromosomal anomalies, among which diploidy appears to be the most common. Therefore, in patients undergoing ICSI who have severely compromised semen parameters, prenatal diagnosis should be performed.

SIGNAL TRANSDUCTION BY NOVEL SPERM CHEMORECEPTORS AS A MECHANISM FOR MATURATION SPERM EGG CHEMOSTAXIS.

L.D. Watsky, M. Rusi*, R. Babin*, G.V. Ronnef*, and S.H. Snyder*. Departments of Neuroscience and Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD.

The identification of transcripts encoding putative olfactory receptors in mammalian germ cells has generated the hypothesis that olfactory receptors may serve a chemosensory role in sperm chemotaxis during fertilization. Using antibodies raised against peptide sequence conserved among known odorant receptors, 35 KD proteins were identified in the membrane fractions of rat, hamster and human sperm. Olfactory receptors were localized by immunofluorescence to the midpiece of the mammalian sperm tail. The midpiece is the site where sperm chemoreceptors may produce the energy required to move. A signaling molecule produced by the egg or female reproductive tract may directly affect locomotion of sperm by activating chemoreceptors on the sperm tail.

Two novel odorant receptor-like partial transcripts were obtained from purified round spermatids by RT-PCR. The partial sequences were used to probe a rat genomic library and two novel subfamilies of the odorant receptor superfamily were identified. The seven transmembrane domain spermad receptors are 35-45 amino acids long and conserved amino acids are identical to the amino acid level to select odorant receptors and are among the most divergent members of the odorant receptor superfamily. RNA protection assays revealed selective expression of the receptors in round spermatids but not in pachytyoxy spermatocytes. In situ hybridization studies localized individual spermad receptor expression to very specific seminiferous tubules. Alternative splicing of the 5'-untranslated regions of the receptors was discovered using RACE-PCR and suggests a mechanism of regulating expression of the novel chemoreceptors.

Antibodies to G-protein receptor kinase 3 (GRK3), B-arrestin 2, and inositol 1,4,5 trisphosphate receptors (IP3Rs) were used in order to determine if these downstream signaling components of the chemoreceptor system may play a role in the chemotaxis of sperm. The proteins GRK3 and B-arrestin 2, implicated in olfactory receptor desensitization, were detected in sperm cytosolic extracts by Western analysis and colocalized with chemoreceptors in elongating spermatids of the testis and in the midpiece of mature sperm. A discrete focus of IP3 staining was detected in the proximal midpiece. Thus, activation of sperm chemoreceptors may produce a receptor burst of calcium mobilization and regulate sperm mobility.

Our results indicate that novel members of the odorant receptor family are selectively expressed in spermatids of the testis. The identification of distinct odorant receptors in rat spermatids, in addition to their colocalization with downstream signaling proteins in the midpiece of mature sperm cells, suggests that these novel proteins may function to mediate sperm-egg chemotaxis.
Calcium flux is required for the acrosome reaction of mammalian sperm. The acrosome reaction is an exocytic event triggered by IP3 binding, which results in a local, dramatic rise in sperm intracellular calcium. Calcium-dependent membrane fusion results in the release of enzymes that facilitate sperm penetration through the zona pellucida during fertilization. We investigated whether mammalian sperm possess inositol 1,4,5-trisphosphate receptors (IP3Rs) and upstream components of the phosphoinositide signaling system that may participate in calcium dynamics. Peptide antibodies colocalized GagII and the IP3 isoform of phospholipase C (PLC) to the anterior acrosomal region of rodent sperm. In situ hybridization using type-specific IP3R riboprobes demonstrated a distinct distribution of IP3R isoforms in the spermatogenic cells of rat testis. Search analysis of the IP3 binding to rat sperm membranes revealed a curvilinear plot with high affinity (Kd=25 nM, Bmax=10 pmol/mg) and low affinity (Kd=1.6 nM, Bmax=350 pmol/mg) sites. Western blotting with a polyclonal antibody directed against purified brain IP3R identified a 250 kD band in IS Triton X-100 extracts of mature rat, hamster, mouse and dog sperm. In each species, IP3R immunostaining localized to the acrosomal cap. Immunoelectron microscopy revealed that IP3Rs specifically decorate the outer acrosomal membrane.

In order to functionally associate IP3Rs with acrosomal calcium flux and the acrosome reaction, we performed reverse 4Ca2+ flux experiments and acrosome reaction assays. Digitonin permeabilized rat sperm loaded Ca2+ in an ATP-dependent manner, in the phosphoinositide signaling system that may participate in calcium dynamics. The presence of 10 µM IP3, but is unaffected by caffeine or ryanodine. This finding suggests inositol 1,4,5-trisphosphate receptors (IP3Rs) and upstream components of the phosphoinositide signalling system that may participate in calcium dynamics. The presence of IP3Rs in the sperm head of mammalian sperm, in addition to the ability to thapsigargin to promote the acrosome reaction, suggests an important role for IP3-gated calcium release in triggering the mammalian acrosome reaction.

14 EFFECT OF SODIUM NITROPRUSSIDE ON SPERM MOTILITY: A DOSE-RESPONSE STUDY

Reactive oxygen species (ROS) are known to inhibit sperm motility and cause damage to the sperm membrane. Antioxidants in semen such as superoxide dismutase (SOD), catalase and glutathione protect the sperm cells from ROS damage. In this study we compared the ROS scavenging efficacy of seminal plasma with other antioxidants towards as in vitro superoxide anion generation system. Superoxide anion was generated using a xanthine oxidase/hypoxanthine assay. Superoxide anion was generated using a xanthine oxidase/hypoxanthine assay. Measurement was made using a luminol induced chemiluminescence assay in a luminometer for 20 minutes. SOD, catalase, ascorbic acid (AA) and pentoxyfylline were compared to a 1:50 dilution of seminal plasma in phosphate buffer (pH 8.7) for their abilities to scavenge superoxide anion. Seminal plasma quenched the chemiluminescence response (peak light units obtained within 3 to 4 minutes of initiation of reaction) in a dose dependent manner ranging from 24% to 75% of the peak. SOD similarly quenched the peak chemiluminescence in a dose-dependent manner showing 36% inhibition at 56 mg/ml to 89% inhibition at 450 mg/ml concentration. Ascorbic acid quenched the peak chemiluminescence completely at a concentration of 1mg/ml. Pentoxyfylline, however, quenched the chemiluminescence to a level of only 11% at the highest dose tested (3 mg/ml). Catalase quenched the peak chemiluminescence only about 2% at the maximum dose (3mg/ml). We conclude that seminal plasma is highly efficient as a scavenger of superoxide anion acting in a similar fashion as SOD and ascorbic acid. Pentoxyfylline and catalase are inefficient in scavenging superoxide anions in this system.
17 DO PEPTIDES STIMULATE SPERM FUNCTION?

Run Wang, Carlos T. Da Ros*, Maryano Barcelos, Hunter Champion,* Suresh C. Sikka, Wayne J. G. Hellstrom, Tulane University School of Medicine, New Orleans, La.

Pituitary adenylate cyclase-activating polypeptide (PACAP) binding sites have been found on spermatozoa tails. Since PACAP increases intracellular cyclic adenosine monophosphate (cAMP) which mediates sperm motion, this study investigated the effects of PACAP27, PACAP38, and VIP on sperm motility and other motion parameters using the CASA system.

Cryopreserved human sperm samples (n = 15) were thawed and then incubated with 140 nMOL/ml of PACAP27, PACAP38, and VIP, respectively. Video sequences were recorded at 0, 30, 60, 120, and 180 min for analysis of sperm motion parameters using the Cell Track Sperm Analysis System. Sperm viability was analyzed by 0.5% eosin stain at the same time.

Our results showed that PACAP27 and VIP had no significant stimulatory effect on sperm motility, viability, and on motion parameters (straight-line velocity, curvilinear velocity, lateral head displacement and mean linearity). Surprisingly, PACAP 38 caused immediate agglutination and death of sperm as demonstrated by sperm motility and viability. This study shows that PACAP27 and VIP have no stimulatory effect on post-thaw sperm function at the given doses. The detrimental effect of PACAP38 on sperm needs further investigation.

18 THE RELATIONSHIP BETWEEN SERUM TESTOSTERONE AND NUTRITIONAL STATUS IN PATIENTS WITH NON- SMALL CELL LUNG CANCER


In the lung cancer population, malnutrition and weight loss are poor prognostic factors with limited successful therapeutic interventions available. In addition, hypogonadism, manifest by reduced testosterone levels has been reported in 43-66% of cancer patients. Testosterone (T) has anabolic effects on the body and is important in maintaining weight and muscle mass. We hypothesize that in patients with unresectable non-small-cell lung cancer (NSCLC) the degree of hypogonadism correlates with nutritional status and quality of life, including sexual functioning. This cross-sectional study of men and women with unresectable NSCLC assessed serum total T, percent ideal body weight, body mass index, upper arm muscle area, and lean body mass. Patients’ gonadal state was assessed by blood analysis of free and total T, FSH, LH, prolactin, and estradiol. Quality of life measures included the Functional Assessment of Cancer Therapy Scale and Derogatis Interview for Sexual Function. Preliminary data from the initial 20 patients (16 male, 4 female) indicate that malnutrition and hypogonadism are prevalent. The mean decline in male patients’ usual body weight was 7% (range -25 to +10%). The mean total T for male patients was 336ng/dl (+/-204). Five of the 15 (33%) male patients had total testosterone levels below the anticipated values for patients of their age and gender. Accrual is ongoing to evaluate the hypothesis that hypogonadism is associated with weight loss and quality of life, and ultimately to evaluate the potential role of testosterone or other anabolic therapy in this population.

19 TRANSESTERIFICATION OF HUMAN SPERM PHOSPHOLIPIDS WITH EXOGENOUS FATTY ACID COENZYME A THIOESTERS. J.G. Ahnert and S. Balogh*, Dept. of Ob/Gyn, Beth Israel Hospital. Boston IVF, Harvard Medical School, Boston, MA.

Human spermatozoa undergo a process of lipid remodeling during migration from the caput to the caudal epididymis. This lipid remodeling involves a decrease in palmitic (16:0) and oleic (18:1) acids and a concomitant increase in arachidonic (20:4) and docosahexaenoic acids (22:6). We recently reported (Mol Reprod Dev, 43:334-46, 1995) that human sperm can incorporate picomolar concentrations of exogenous fatty acids and that this incorporation is mediated by the phospholipid remodeling pathway. The objective of this study was to investigate the ability of human sperm to transensterify micromolar concentrations of fatty acids into the phospholipid pool from exogenous fatty acid CoA thioesters. A total of 12 semen samples were obtained from 12 healthy sperm donors. All samples were normal by WHO criteria. The seminal plasma was removed by centrifugation at 600g for 8 min and the resulting pellet resuspended in BWW medium containing 5mg/ml of BSA. The sperm suspensions were then incubated at 37°C in the presence of 0.2-5 µM oleoyl CoA for up to 4 h. Aliquots of the sperm suspensions were removed at 1 h intervals, centrifuged at 800g for 8 min and the resulting pellet extracted with 20 vol of chloroform-methanol 1:1. The phospholipid fraction was then isolated by aminopropyl column chromatography, the fatty acids methylated using BF3-methanol, and the resulting fatty acid methyl esters analyzed by gas chromatography. Incorporation of oleic acid into the phospholipid pool followed first order kinetics at oleoyl CoA concentrations between 0.2 and 2 µM and zero order kinetics at concentrations > 2 µM. The mean concentration of phospholipid 18:1, 20:4 and 22:6 in control samples was 15 ± 2, 9 ± 2 and 52 ± 7 µg/106 cells, respectively, and that obtained after 4 h of incubation in the presence of 2 µM oleoyl CoA was 35 ± 4, 5 ± 0.8 and 28 ± 3 µg/106 cells, respectively. These differences were statistically significant (P < 0.001). These results indicate that human sperm have (i) the ability to transensterify micromolar concentrations of fatty acids into the phospholipid pool from exogenous fatty acid CoA thioesters, and that (ii) transensterification of fatty acids from exogenous fatty acid CoA thioesters results in a decrease in endogenous phospholipid 20:4 and 22:6 suggesting that this incorporation is mediated by the phospholipid remodeling pathway.

20 A ROLE FOR SGP-I IN BINDING OF SPERM TO EGGS


SGP-1 is a highly conserved molecule secreted by Sertoli cells and epithelial cells of the ecurrent ducts in rats, bound to sperm, but of unknown function, the protein sequence is identical to prosaposin. Compared to fresh sperm, frozen-thawed rooster sperm are of low fertility and a low percentage bind in vitro to an egg membrane substrate (assembly modified from BJR 80 Suppl 1:128. 1994). A semi-purified extract (UPSEBP = native bioactive molecule) prepared from supernatant of thawed rooster sperm was obtained. Inhibition studies led to probing Western blots with polyclonal antibody against rat SGP-1, a - 10kD band stained. Pre-treating semi-purified UPSEBP with antibody before use to treat sperm eliminated binding. Studies with saposins A-D, antibodies against saposins, and synthetic sequences for portions of rat SGP-I led to conclusions that UPSEBP was not a known saposin, but was a fragment of SGP-I. A synthetic 60-Amino acid sequence with high bioactivity (FertPlus™) was identified, based on enhanced in vitro binding of rooster to the egg-membrane substrate. When sperm from individual roosters were treated with either UPSEBP or FertPlus™ peptide and used for AI, both maximum fertility and duration of fertile life span increased. We speculate that UPSEBP is a proteolytic cleavage product of SGP-I, formation of which is facilitated by an alternative post-translational folding and proteolysis of SGP-I relative those of prosaposin. We concluded that UPSEBP is highly conserved across species, ubiquitous in semen from common animals, can be eluted from sperm by freeze-thawing, normally becomes bound to the sperm plasma membrane over the head, facilitates initial sperm-egg binding, and may be in suboptimal amounts on sperm from some males. Deficiencies can be restored by in vitro treatment of sperm with FertPlus™ peptide prior to AI. MD Griswold & JS O'Brien provided reagents. USDA92-92202-073
To investigate proteins involved in the fertilization process male rats were treated for 10 days with the antifertility agent ornidazole at a dosage of 400 mg/kg. This short time frame was chosen to create a large amount of sperm-free epididymis. CASA analysis revealed a MW of 20,420 Da. Digestion of the protein and partial sequence analysis of 17 amino acids demonstrated no homology to any sequenced product. The protein is not found in control animals. Its molecular weight was ~25 kDa with a pl of ~5.8. The intensity of staining the protein was present in greater amounts in the cauda than corpus fluid. Incubation of tissue from the epididymal caudal, corpus and cauda regions of the epididymis by tubule cannulation and perfusion generated a 50% decrease in ATP concentration, and a concomitant -60% decrease in reduced glutathione levels and ~100% increase in oxidized glutathione levels compared to controls. These data demonstrate a biological response to superoxide anion and hydrogen peroxide confirming the generation of oxygen radicals within the experimental tissue. RNase protection assays using [32P]-labeled probes of the 5'UTR of GGT mRNA II, III, IV demonstrated a ~70% increase in the expression of GGT mRNA II yet only a ~20% increase in the expression of GGT mRNA III and IV compared to controls under these same conditions. These results support the hypothesis that expression of GGT mRNA II, in particular, is regulated by the oxidative status of the initial segment in the epididymis. This study contributes to the growing body of evidence that the epididymis plays a critical role in protection of sperm from oxygen radicals and other xenobiotics. Further studies are underway to examine the mechanisms by which oxidative stress upregulates GGT mRNA II. Supported by NIH grant HD32979 (BTH), NIH P30-HD28934, and training grant NIH T32-DK07642 (DBR).

FasL is a type II transmembrane protein of the tumor necrosis factor (TNF) family that has been extensively studied in lymphoid tissue. It is associated with down-regulation of the immune response by inducing apoptosis of activated lymphocytes. Although the RNA message for FasL has been identified in the rat testis, its protein expression and cellular distribution has not yet been reported. Normal and ischemic testes and normal epididymides were fixed in 10% NBF and paraffin imbedded. Sections (7µm) were immunostained with the ABC (Vector Labs) immunoperoxidase bridge utilizing rabbit polyclonal antibody for FasL (Santa Cruz Biotechnologies) and DAB and TB as chromogenic substrates. Controls included first antibody deletion, antibody absorption and splenic tissue. Immunostaining was apparent on basal compartment germ cells, Sertoli cells and epididymal epithelium. Generation of reaction product appeared greater in the ischemic testis when compared to the normal testis. Results suggest that cell death in the testis, induced by ischemia, may be associated with the FasL apoptotic pathway. Additionally, the expression of FasL in the testis and the epididymis suggests a mechanism of immune regulation in these two organs.

22 IMMUNOEXPRESSION OF FAS (CD95) LIGAND (FasL) IN THE RAT TESTIS AND EPIDIDYMIS F.C. Griffin and D.F. Cameron, University of South Florida College of Medicine, Tampa, FL 33612

FasL is a type II transmembrane protein of the tumor necrosis factor (TNF) family that has been extensively studied in lymphoid tissue. It is associated with down-regulation of the immune response by inducing apoptosis of activated lymphocytes. Although the RNA message for FasL has been identified in the rat testis, its protein expression and cellular distribution has not yet been reported. Normal and ischemic testes and normal epididymides were fixed in 10% NBF and paraffin imbedded. Sections (7µm) were immunostained with the ABC (Vector Labs) immunoperoxidase bridge utilizing rabbit polyclonal antibody for FasL (Santa Cruz Biotechnologies) and DAB and TB as chromogenic substrates. Controls included first antibody deletion, antibody absorption and splenic tissue. Immunostaining was apparent on basal compartment germ cells, Sertoli cells and epididymal epithelium. Generation of reaction product appeared greater in the ischemic testis when compared to the normal testis. Results suggest that cell death in the testis, induced by ischemia, may be associated with the FasL apoptotic pathway. Additionally, the expression of FasL in the testis and the epididymis suggests a mechanism of immune regulation in these two organs.


Oxygen radicals have a powerful cytotoxic effect on spermatozoa and have been implicated in sperm dysfunction and male infertility. Recent evidence suggests that the epididymis utilizes the antioxidant glutathione to protect maturing sperm against oxidative stress. One enzyme essential to the metabolism of glutathione is gamma-glutamyl transpeptidase (GGT). We have previously shown that multiple forms of GGT mRNA (II-IV) are differentially expressed in the epididymis and that they are regulated by androgens and/or testicular factor(s). The aims of this study were (1) to establish in vitro conditions in which oxygen radicals are generated, and (2) to determine if oxidative stress regulates the expression of GGT mRNAs II-IV in the initial segment of the epididymis. Initial segments were collected from adult male rats and incubated for 1.5h, 3.5h and 6.5h in culture media to which oxygen radical-generating compounds, hypoxanthine and xanthine oxidase, were added in varying concentrations. By 6.5h incubation of tissue in high oxidizing conditions caused a ~50% decrease in ATP concentration, and a concomitant ~60% decrease in reduced glutathione levels and ~100% increase in oxidized glutathione levels compared to controls. These data demonstrate a biological response to superoxide anion and hydrogen peroxide confirming the generation of oxygen radicals within the experimental tissue. RNase protection assays using [32P]-labeled probes of the 5'UTR of GGT mRNA II, III, IV demonstrated a ~70% increase in the expression of GGT mRNA II yet only a ~20% increase in the expression of GGT mRNA III and IV compared to controls under these same conditions. These results support the hypothesis that expression of GGT mRNA II, in particular, is regulated by the oxidative status of the initial segment in the epididymis. This study contributes to the growing body of evidence that the epididymis plays a critical role in protection of sperm from oxygen radicals and other xenobiotics. Further studies are underway to examine the mechanisms by which oxidative stress upregulates GGT mRNA II. Supported by NIH grant HD32979 (BTH), NIH P30-HD28934, and training grant NIH T32-DK07642 (DBR).


The promoter region of rat gamma glutamyl transpeptidase (GGT) mRNA-IV contains at least five exact matched PEA3 motifs (5'-AGGAAG-3'). Our previous studies have shown that multiple forms of GGT mRNA (II-IV) are expressed in the epididymis and one form, mRNA-IV, is highly expressed in the initial segment and is regulated by testicular factors. We hypothesize that PEA3 functions to regulate rat GGT mRNA-IV expression and that its expression is also regulated by testicular factors. The goals of this study were (1) to examine PEA3 protein level and mRNA expression along the epididymal duct; (2) to determine DNA binding activity of PEA3 to promoter IV of the rat GGT gene; and (3) to determine if expression of PEA3 mRNA is regulated by testicular factors. Western blot analysis showed that a 62 kDa protein was detected in the nuclear extracts from the initial segment in greater levels compared to the distal epididymal regions. Electrophoretic mobility shift assay (EMSA) showed that nuclear extract isolated from the initial segment specifically bound to the native PEA3 motif derived from the promoter region of GGT mRNA IV. This DNA protein complex contained the 62 kDa PEA3 protein as demonstrated by EMSA and Western analysis using PEA3 mAbs and a cationized PEA3 motif probe. Northern blot analysis and RNase protection assays showed that a 2.4 kb PEA3 mRNA was highly expressed in the initial segment and its expression was regulated by testicular factors. PEA3 mRNA levels were reduced to 68% and 85% of sham-operated control levels after 12 and 24 h efferent duct ligation respectively. Our findings support the hypothesis that PEA3 is involved in the regulation of rat GGT mRNA-IV in the initial segment, and that its expression is regulated by testicular factors. Further work is underway to identify the testicular factors and the mechanisms by which the expression of PEA3 and GGT genes are regulated in the epididymis.

Supported by N.I.H. grants HD18257 & HD32979 (BTH), P30-HD28934, and training grant T32-DK07642 (DBR).
THE STATUS OF CAUDA EPIDIDYMAL SPERM DNA FOLLOWING PARTIAL SYMPATHETIC DENERVATION. M. Schwartz* and D.D. Ricker, Department of Biological Science, York College of Pennsylvania. York, PA.

Previous studies in the rat have demonstrated that the loss of cauda epididymal sympathetic innervation via the Inferior Mesenteric Ganglion (IMG) adversely affects sperm transport, maturation, and storage. Moreover, our lab has shown that while cauda epididymal sperm from IMG-denervated rats can fertilize oocytes, the resulting embryos fail to develop successfully. The objective of this study was to examine whether this impaired embryo development was related to changes in cauda sperm DNA following the loss of epididymal sympathetic innervation. For this investigation, we utilized adult male Sprague-Dawley rats (n=16 total) which had undergone either a sham (control) procedure or surgical IMG removal 1 week previously. Spermatozoa were collected from excised cauda epididymal tubules and were subjected to three distinct experiments in order to detect potential DNA breaks. Acridine Orange fluorescent labeling, Apoptag immunostaining, and a DNA fragmentation assay. The results of each order to detect potential DNA breaks: Acridine Orange fluorescent labeling, Hoechst 33258 and nuclear area was measured under fluorescence microscopy.

27 URINARY CREATINE AS A BIOMARKER FOR LEAD INDUCED TESTICULAR DAMAGE. S.R. Skaetter*, W.J. Moorman*, D.D. Shampack*, and S.M. Schrader, NIOSH, 4676 Columbia Parkway, Cincinnati, OH 45226

In recent years there has been a growing concern about environmental and industrial exposure to a wide variety of chemicals and/or compounds which affect the male reproductive system. Several studies have been the most common means for determining a male’s ability to produce viable sperm capable of fertilizing an ovum. These studies normally evaluate sperm parameters (count, motility, morphology) and biochemistry. Timbrell et al. (Tox. & Environ. News, 1(1), 4-14, 1994) states that the biomarkers for toxic effects on the male reproductive system are mostly invasive or the samples are not easy to collect and suggests that a urinary biomarker for testicular damage would be extremely valuable. While urinary creatine is not traditionally measured as a marker for testicular damage, an increase in urinary creatine has been reported to coincide with testicular damage induced by cadmium (Nicholson et al. Mol. Pharmacol, 36, 398-404, 1989) and 2-methoxyestradiol (Timbrell et al. Int. Symp. Health Haz. Gylcoc Esters, Nancy, France, 1994). However, no reports of an increase in urinary creatine levels due to lead induced testicular damage have been reported. As part of a larger project to study the reproductive effects of lead in the rabbit urinary analyte, creatine, its ratio, and histological evaluation of the testis was conducted to determine if creatine might serve as a useful biomarker of lead induced testicular damage. Rabbits were given subcutaneous injections of lead acetate in 5% dextrose to achieve dose levels of 0.0 µg/dl, 20 µg/dl, 40 µg/dl, and 80 µg/dl. Histologic evaluation of the testes showed altered spermiogenesis characterized by a dose response increase in defective elongate spermatids. However, a decrease in testicular weight was not found. Assays were performed to determine if there was a dose response increase in creatine and creatinine levels. A statistical increase in urinary creatine (Ps 0.5) was found in the highest (80 µg/dl) dose group. Neither creatinine (P= 0.8327) nor the ratio of creatine/creatinine (P= 0.1243) was altered with lead dosing. Given published reports of increased urinary creatine coinciding with testicular damage, increased urinary creatine levels in male rabbits may be associated with lead induced testicular damage at high exposure levels.


Oxidative field studies utilizing semen analysis are often plagued with low participation rates. Requiring workers to provide semen samples by masturbation has been blamed for this poor participation (Levine et al., JOM 25:391, 1983). Silastic condoms have been marketed as an alternative to masturbation for the collection of a viable semen sample for clinical evaluations. These condoms were evaluated in a Health Canada study designed to measure pesticide exposures of the applicator and family members on Ontario farms. Semen and urine samples were collected from participants to analyze for pesticide residues. As all of the farmers in this study were married, the silastic condom could be used to collect the semen during normal marital intercourse. Twelve interviewers received two days of training on the study design, interviewing techniques, and instructions for participants on the collection of biological samples. Potential participants were telephoned to arrange a visit to the farm to describe the study. Informed consent was obtained and the collection devices were left with the family. The couple was requested to collect semen in the silastic condom within 48 hours of exposure to the pesticides of interest. The condom was then tied, placed in the pocket provided, and frozen. The samples were picked up by the study team within 4 days and transported to the University of Guelph for pesticide analyses. Three hundred and twenty-nine families were contacted to arrange a home visit. At this stage, 17% refused to participate and 4% were found to be ineligible (not planning to spray pesticides under study). Of the 258 families that were visited, 20 were declared ineligible, 215 agreed to participate in the study (89%) with 186 men (78%) agreeing to provide a semen sample. Subsequently 51 families became ineligible because they did not spray the chemicals of interest during the study period. Of the eligible families that agreed to participate, the compliance rate for urine and semen was 70% and 58%, respectively. These data suggest that the use of the silastic condom for future occupational field studies may improve participation rates.

(Grants: FISS 94-399 and DGICYT PB 93-370)
SERTOLI CELLS IN CULTURE AND mRNA DIFFERENTIAL DISPLAY PROVIDE A SENSITIVE EARLY WARNING ASSAY SYSTEM TO DETECT XENOBIOTICS

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We have developed a sensitive early detection method to investigate the effects of low doses of potential testicular toxicants long before decreases in sperm count would be seen. Sertoli cells were used as an early warning system to monitor morphological and biochemical changes induced by two different xenobiotics—cadmium acetate and polychlorinated biphenyls (PCBs). Sertoli cells begin to round, vacuolize and detach from their substrate within 24 hours of culture in the presence of cadmium at concentrations of 0.5 to 1.0 µM. The mixture of PCBs used in the present study was shown to have estrogenic activity, which causes reproductive disorders including reduced fertility. Sertoli cells cultured for 24 hours in the presence of the PCBs (3'3' 4'4' tetrachlorobiphenyl, 2'2' 4'6' pentachlorobiphenyl, and 2'2' 3'3' 4'5' 5'6' nonachlorobiphenyl) at concentrations of 1.0-2.0 µM showed cellular enlargement. Despite their changes in morphology, no reduction in Sertoli cell viability was seen at any of the concentrations or time points studied for either toxicant. Using mRNA differential display, a number of novel cDNAs were detected when cells were cultured either with cadmium or PCBs showing that changes in gene expression accompany the changes in Sertoli cell structure. We propose that Sertoli cells in culture and mRNA differential display provide a sensitive morphological and biochemical assay system to detect early direct effects of low doses of toxicants on mammalian testicular cells.
33 DECLINE IN LEYDIG CELL VOLUME IN A GROUP OF NORTH AMERICAN MEN OVER THE LAST 16 YEARS. L. Johnson, S. W. Brown, and J.J. Barnard. Dept. of Vet. Anatomy & Public Hlth., Texas Veterinary Medical Center, Texas A&M University, College Station, TX. Dept. of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.

Reports of diminished seminal quality including reduced seminal volume in recent years have sparked interest in the steroidogenic potential in human males. A direct relationship exists between Leydig cell organelle content and spermatogenic potential in humans. Changes in volume of Leydig cells/man or size of individual Leydig cells may illustrate a man's steroidogenic potential and may indicate a means for a change in seminal quality of men in recent years. The objective was to determine if the volume of Leydig cells have declined during a 16-year period. Testes, obtained at autopsy in Dallas, were fixed with glutaraldehyde, further fixed with osmium, embedded in Epon, and evaluated by stereology for volume density of Leydig cells and Leydig cell nuclei. For years '80, '86, and '96, respectively, fairly large numbers of men (13, 8, and 21) of similar ages (31 ± 3, 31 ± 1, and 28 ± 1 yrs.) were used. Estimated paired testicular parenchymal weight (46 ± 3, 43 ± 7, and 42 ± 2 g), volume density of testicular interstitium (34 ± 2, 29 ± 3, and 32 ± 2 %), volume density of Leydig cells (5.7 ± 0.4, 5.0 ± 0.6, and 4.8 ± 0.3 %) and number of Leydig cells/man (529 ± 38, 438 ± 50, and 337 ± 9 f) were greater (P<0.05) in '80 than '86 or '96. Based on direct measurement of steroidogenic Leydig cell volume of these North American men, there appears to have been a decline in steroidogenic potential which could contribute to a reduction in seminal volume during the last 16 yrs. NIH K04 AG 00464-05

35 AGE - RELATED ALTERATIONS IN PLASMA NITRITE AND NITRATE LEVELS IN IMPOTENT MEN. M. Rajasekaran, W.J. Hellstrom, S.C. Sikka, Tulane University School of Medicine, New Orleans, LA.

Nitric oxide (NO), synthesized by nitric oxide synthase (NOS) is a non-adrenergic, non-cholinergic mediator of penile erection. Little is known about the age-related changes in relation to NO production in impotent men. Nitrite and nitrate are the stable end-products of the NO pathway. Plasma levels of these products have been used as markers for the production of nitric oxide in a variety of clinical conditions. NO-producing activity has been found to decrease with aging in several animal models. In the present study, the plasma levels of nitrite and nitrate of impotent patients of two age groups (group I: 50-60 years and group II: 60-70 years) were evaluated. Blood samples (n = 10) were obtained during penile prosthesis implantation and the plasma was stored at -70 C. Nitrate was enzymatically converted to nitrite using nitrate reductase (from Aspergillus spp). Samples were deproteinized with protamine sulfate (10 mg/mL) and nitrite was colorimetrically measured using Griess reagent (0.1% sulfanilamide + 0.1% N-1-naphthylethenediamine in 5% phosphoric acid). In group I, the mean (±SEM) nitrite + nitrate concentration (µ M) was 19.18 ± 4.83 while the mean of group II patients was 10.01 ± 3.87. This 2-fold decrease in NO-end products in plasma of group II patients suggests that age related reduction in NO production may contribute towards the altered penile hemodynamics in this impotent population. This work was supported in part by an AFUD/Pharmacia-Upjohn Scholarship.

36 PENILE ERECTION INDUCED BY TRANSCUTANEOUS ADMINISTRATION OF NOVEL NITRIC OXIDE DONORS. Wayne J. G. Hellstrom, Run Wang, Hunter C. Champion®, Suresh C. Sikka, Larry K. Keeffer*, Paul Doherty, Tulane University School of Medicine, New Orleans, LA, National Cancer Institute, Frederick, MD, VIVUS, Inc. Menlo Park, CA

Our earlier studies have demonstrated that intracavernosal injection of nitric oxide (NO) donors can induce penile erection. This study investigates the effect on penile erection in an in-vivo feline model by the transurethral use of the novel NO donors: Proli/NO, (CH3)3 CCoo·Na · N [N (O) NO] · Na• and piperazine, (CH3),NH2 · N ((0) NO`). This study supports the transurethral administration of these novel NO donors in the pharmacologic treatments of erectile dysfunction.


Proadrenomedullin N-terminal 20 peptide (PAMP) is a novel 20 amino acid peptide encoded by the adrenomedullin gene. PAMP has been shown to have direct, cAMP-mediated vasodilator activity in the cat. The present study was undertaken to investigate the ability of intracavernosal injections of PAMP to induce penile erection in the cat. PAMP was delivered by a 30-gauge needle intracavernosally. 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. The reference drug combination (1.65 mg papaverine, 25µg phenolamine and 0.5µg PGE1) was injected intracavernosally at the end of each experiment for control comparison. Intracavernosal injection of PAMP caused penile erection in a dose-dependent manner in cats. The maximal effect on intracavernous pressure was 70% of that obtained by the intracavernous injection of the standard drug combination. The maximal increase in penile length (23.6 ± 0.8 mm from a baseline of 16.7 ± 0.9 mm) was comparable to that caused by the standard drug combination. The duration of the maximal pressure and the total duration of the drug effect caused by PAMP was shorter than that caused by the standard reference combination. Intracavernous injections of PAMP did not significantly decrease systemic blood pressure whereas the standard reference drug combination decreased blood pressure by 33.6 ± 17.2 mmHg (p<0.01). The present study supports the potential clinical investigation of PAMP to induce penile erection via intracavernous injection.
37 a.-BLOCKERS ENHANCE PENILE ERECTION EFFECT OF PGE1 IN CATS
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This was designed to investigate the effect of TSE using a combination of PGE1 and a-blockers to induce penile erection in cats.

Intracavernosal injection of galanin (250 µg) was performed to induce penile erection in a close-dependent manner. The maximal effect on intracavernosal pressure was observed at doses of 200 µg galanin (98.1 ± 19.6 mmHg from a baseline of 17.1 ± 3.2 mmHg, p < 0.01), 200 µg Prazosin (77.1 ± 12.3 mmHg from a baseline of 16.4 ± 1.6 mmHg, p < 0.01), and 10 µg Tamsulosin (82.5 ± 18.6 mmHg from a baseline of 19.2 ± 2.8 mmHg, p < 0.01). The penile length was maximally increased by the same doses of galanin (25.3 ± 1.1 mm from a baseline of 17.5 ± 0.6 mm, p < 0.01), Prazosin (25.3 ± 1.5 mm from a baseline of 17.9 ± 1.0 mm, p < 0.01), and Tamsulosin (25.3 ± 1.1 mm from a baseline of 17.5 ± 0.6 mm, p < 0.01). The erection response induced by these a-blockers combined with PGE1 was significantly enhanced by galanin when compared to the standard drug combination (1.65 mg papaverine, 25 µg phenolamine and 0.5 µg PGE1). Intracavernosal injection of galanin caused penile erection in a close-dependent manner in cats. The maximal effect on intracavernosal pressure (98.4 ± 13.1 mmHg from a baseline of 16.2 ± 1.8 mmHg) and penile length (25.3 ± 1.8 mm from a baseline of 17.7 ± 0.7 mm) were comparable to that caused by the standard drug combination. The duration of the maximal pressure and the total duration of the drug effect caused by galanin was more abbreviated than that caused by the standard reference combination. Intracavernosal injections of galanin did not significantly decrease systemic blood pressure whereas the standard drug combination decreased systemic blood pressure by 48.6 ± 11.4 mmHg (p < 0.01).

39 GALANIN INDUCES PENILE ERECTION IN THE CAT

Galanin is a 29 amino acid peptide that is widely distributed in both the central and peripheral nervous systems. Recently, the peptide has been shown to be present in penile neurons of the rat and human. However, little if anything is known about the role of galanin in mediating penile erection. The present study was undertaken to investigate the ability of intracavernosal injections of galanin to induce penile erection in the cat.

Galanin was delivered by a 20-gauge needle intracavernosally. The response was characterized by changes in intracavernosal pressure, duration of the drug effect, change in penile length, and alterations to the systemic arterial blood pressure. The reference drug combination (1.65 mg papaverine, 25 µg phenolamine and 0.5 µg PGE1) was injected intracavernosally after each experiment for control comparison.

Intracavernosal injection of galanin caused penile erection in a close-dependent manner in cats. The maximal effect on intracavernosal pressure (98.4 ± 13.1 mmHg from a baseline of 16.2 ± 1.8 mmHg) and penile length (25.3 ± 1.8 mm from a baseline of 17.7 ± 0.7 mm) were comparable to that caused by the standard drug combination. Intracavernosal injections of galanin did not significantly decrease systemic blood pressure whereas the standard drug combination decreased systemic blood pressure by 48.6 ± 11.4 mmHg (p < 0.01).

Results of the present study support the hypothesis that galanin may have a role in mediating penile erection. Furthermore, the present study supports the potential clinical investigation of galanin to induce penile erection via intracavernosal injection.
41 ASSESSMENT OF THE MALE ACCESSORY GLANDS OF INFERTILE MEN WITH KALLIKREIN hK2 IN SEMINAL PLASMA.
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The human prostate expresses the 3 known kallikreins namely hK1, hK2 and hK3 (Prostate Specific Antigen). While the roles and/or variations of hK1 and hK3 in seminal plasma have been previously described, those of hK2 are totally unknown. Thus, the availability of the purified kallikrein hK2 and the development of highly specific antibodies against the protein (no cross-reactivity with other prostatic proteins) allowed us to set up an ELISA assay for the measurement of hK2 in biological fluids. This assay was used to add complementary information or to ascertain observations, based on Western analysis, indicating impressive variations in the profile of free or truncated hK2 (secreted by the prostate) and the hK2 bound to Protein C Inhibitor (PCI) in seminal plasma (SP). PCI is a specific product of the seminal vesicles. To achieve this goal, four groups of subjects were selected: fertile men (FM), vasectomized subjects (V), oligoasthenoteratozoospermic patients (OAT) and azoospermic men (A). hK2 was expressed in µg/ml of SP. In FM (N=32), V (N=36), OAT (N=34) and A (N=33), hK2 was respectively 2.2±0.9 (SD), 1.8±1.0 (SD), 1.8±1.2 (SD) and 1.7±1.4 (SD). No significant difference was observed between FM and infertile subjects. In conclusion, the study of the secretory function of either seminal vesicles or prostate appears much more discriminatory when using a Western blotting technique to appreciate PCI and/or hK2 variations. Our ELISA assay provides only valuable information on the sum of hK2 and hK2-PCI in SP. By contrast with hK3 (J. Androl. 16, No 6, 1995), hK2 is not decreased in infertile men.
Supported by MRC grant.

42 THE EFFECTS OF SPINAL CORD INJURY ON TRPM AND ANDROGEN RECEPTOR mRNA IN THE PROSTATE OF THE RAT H.F.S. Huang, M.T. Li, T.A. Linsenmeyer, J.E. Ottmiller, L.M. Pogach and R. J. Irwin, Departments of Surgery, Physical Medicine/Rehabilitation, Neuroscience and Medicine, UMD-New Jersey Medical School, Newark; Kessler Institute of Rehabilitation, West Orange; and V. A. Medical Center, East Orange, New Jersey.

In men, sperm motility is usually abnormal after spinal cord injury (SCI). This could result from abnormal spermatogenesis, or due to changes in biochemical properties of the seminal fluid. In this study, we examined the expression of mRNA for testosterone repressed prostate message (TRPM-2) and androgen receptor (AR) in the prostate of rats after surgical transection of the spinal cord at the levels of T9 or L1 vertebra. SCI resulted in an acute decrease in prostate weight and an increase (P<0.05) in steady state level of prostate TRPM-2 mRNA that concurred with a decrease in serum testosterone. In contrary, there was a transient but significant decrease (P<0.05) in the steady state level of AR mRNA. Although normal serum testosterone concentrations and prostate AR mRNA levels have subsequently been restored, prostate weights remained persistently lower in SCI rats than sham controls for at least 3 months. Concomitantly, the level of TRPM-2 mRNA remained elevated as late as 6 months after the injury. Morphometric analysis revealed a decrease in the height of prostate epithelial cells in the SCI rats, and evidences of abnormal epithelial morphology and possibly cell death were also noted in some animals. These results demonstrate a prolonged effect of SCI on prostate function. These findings suggest that male accessory sex glands may be compromised after SCI. These changes may affect biochemical properties of the seminal plasma and may provide some explanation for abnormal sperm motility seen in the semen of SCI men.

43 ISOLATION AND PURIFICATION OF BASAL AND SECRETORY EPITHELIAL CELLS FROM THE RAT VENTRAL PROSTATE N. Ravindranath and M. Dym, Department of Cell Biology, Georgetown University Medical Center, Washington, DC.

The epithelium of the prostate consists of basal, secretory, and endocrine-paracrine cells. It is hypothesized that the basal cells are the stem cells which can either renew themselves to form new basal cells, or differentiate into secretory and endocrine-paracrine cells. Our objective was to isolate and characterize a pure population of basal cells. Minced pieces of ventral prostates from four adult rats were suspended in DMEM/F12 medium containing collagenase (1 mg/ml) and DNase (1 µg/ml), and incubated at 37°C for 20 min in a shaking water bath operated at 100 cycles/min. Large sheets of glandular epithelium, which separated from stromal cells, were sedimented and resuspended in DMEM/F12 containing collagenase (1 mg/ml), hyaluronidase (1 mg/ml), and DNase (1 µg/ml), and incubated for 30 min at 37°C. The dispersed clumps of epithelial cells were plated in DMEM/F12 plus 10% FBS. After incubation overnight at 37°C, the floating epithelial cell clumps were removed and subjected to a 0.05% trypsin-EDTA solution for 5 minutes. The dispersed cells were then filtered through 80-µM and 40-µM nylon mesh successively, and separated by sedimentation velocity at unit gravity. hK3 is not decreased in infertile men.

5.3 INTRAPROSTATIC INJECTION OF ZINC SALT AS A TREATMENT FOR BENIGN PROSTATIC HYPERPLASIA.
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Fifty percent of men over the age of 50 years have some degree of hyperplastic enlargement of the prostate while 95% of men will experience symptoms related to prostate enlargement by the time they reach the age of 85 years. Twenty-five percent of all patients seen by urologists suffer from benign prostatic hyperplasia (BPH). Therefore, we studied intraprostatic injection of zinc salt as a treatment for BPH (J. U.S. Patent Nos. 4,964,688; 5,071,658; 5,234,698; Andrologia 25:369, 1993). A pilot clinical study was conducted in 34 patients who had been diagnosed with BPH. One milliliter of 100 mg zinc salt was injected into each lateral lobe of the prostate of patients with BPH. All of the patients experienced a sensation of swelling at the area of injection for 1-4 days. No other side effects were observed. The results of the pilot study indicated significant decrease in symptoms of obstruction, an increase in urinary flow, and a decrease in prostatic volume. The average of the American Urology Association (AUA) Symptom Index of all injected patients decreased from 27.8 to 11.5 four weeks after injection. Future clinical investigations will be designed and include a longer post-injection follow-up period.
Current treatment for advanced prostate cancer uses androgen deprivation therapy (ADT) to induce a hypogonadal state. We hypothesized that this therapy would cause a decrease in bone mineral density (BMD), leading to accelerated bone loss and an increased risk of fracture. Osteopenia, a condition in which BMD is between 1 and 2.5 standard deviations below the young adult mean, is considered prognostic of future fracture risk. In the present cross-sectional study we examined the femoral neck and lumbar spine BMD of 13 men (age 57 to 80 yrs., mean 72 ± 6 yrs.) with prostate cancer who had undergone ADT for at least 18 months. The spinal BMD values for 6 of those men were unusable due to reactive sclerosis. The mean spinal BMD of patients without sclerotic changes (mean ± SD 0.888 ± 0.123 g/cm², n=7) was significantly below that of age-matched normals (p < 0.02). Five of these 7 men (71%) had osteopenia in the femoral neck (0.720 ± 0.082 g/cm²) was slightly lower than age-matched normals (p < 0.1). Deoxypyridinoline (DPD) and osteocalcin, markers of bone resorption and formation respectively, have been used clinically to determine the status of bone turnover. In this study the mean DPD concentration was 12.85 ± 4.58 nmol DPD/mmol creatinine (normal range 3.0 - 10.7) while the mean osteocalcin concentration was 9.3 ± 2.3 ng/ml (normal range 5.1 - 16.9). These results suggest that androgen deprivation therapy in patients with prostate cancer may augment the rate of bone loss and increase the risk of fracture, resulting in significant adverse effects on quality of life and morbidity.

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THE REGULATION OF PROLACTIN AND EGF RECEPTOR mRNAs IN A HUMAN PROSTATE CANCER CELL LINE (LNCaP) BY LHRRH

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Prolactin receptors (PRL-R), members of cytokine-hematopoietin receptor superfamily in which EGF-R is also included, mediate PRL biological functions, including cell proliferation. LHRRH analogs, known as anti-prostate cancer drugs, suppress androgen levels through the pituitary-testicular axis. However, LHRRH agonists were also shown to have direct anti-proliferative effects on prostate cancer cell lines. To ascertain whether LHRRH can directly regulate mRNA levels of PRL-R and EGF-R in androgen-dependent LNCaP cells, treatments were compared. Results: PRL-R mRNA levels increased by 65% at 3 hr incubation with 10⁻⁷M LHRRH, and by 50% at 6 hr with 10⁻⁹M LHRRH, compared to controls (p<0.05). This up-regulation was mainly attributed to the increase of the short form PRL-R since there was no significant change in the long form PRL-R mRNA. In contrast, EGF-R mRNA was down-regulated by 49% (p<0.05) at 12 hr incubation with LHRRH (10⁻⁷M). Conclusions: 1) PRL-R mRNA was shown for the first time to be expressed in LNCaP cells, of which the short form was up-regulated by LHRRH. These results encourage studies on the role of PRL in prostate cancer. 2) The down-regulation of EGF-R mRNA by LHRRH may reflect its anti-proliferative action on prostatic tumor cells.

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THE CYNOOMOLUS MONKEY PROSTATE UNDER PHYSIOLOGICAL AND HYPOGONADAL CONDITIONS. AN ULTRASONOGRAPHIC STUDY

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Objective: Although the prostate of the cynomolgus monkey has been shown to be a particularly suitable model for the human, in vivo data on this organ are hardly available. In the present work we apply transrectal ultrasonography for monitoring prostate size longitudinally and investigate the dynamics of prostate size under physiological and hypogonadal conditions. Materials and Methods: Five adult intact and 7 long-term castrated monkeys were analysed repeatedly for assessment of the feasibility and variability of the method. In addition, cross-sectional evaluation was performed on 30 adult monkeys of various ages and on 7 long-term castrated monkeys. Finally, the vehicle (n=5) controlled effects of androgen deprivation treatment with the GnRH antagonist cetorexil (n=4) for 25 days followed by semicastration at day 16 and full castration on day 25 were examined and prostate size was followed thereafter. Results: The monkey prostate shows an ellipsoid shape and ultrasonic appearance similar to the human prostate. Variability in 5 intact monkeys (mean±SE= 4.6±0.35 ml) and 7 long-term castrated monkeys (0.8±0.08 ml) was 8.9% and 5.6% respectively. In intact animals a linear correlation between age and prostate size was found (p<0.0004). Prostate size in castrated animals was reduced (p<0.0005) compared to intact animals. In the experimental study, GnRH antagonist led to a significant decrease of prostate volume and testosterone levels compared to the vehicle group, while semicastration had no influence. Following removal of the second testis a further decrease of prostate volume was seen (p<0.0001). When compared, castration and GnRH antagonist treatment induced a similar decrease of prostate volume over time. Conclusion: Transrectal ultrasonography provides a feasible and reproducible approach for determination of prostate size in cynomologus monkeys. Castration and GnRH antagonist treatment are equally effective in reducing prostate size. References: 1) Habenschütz U.F., M.F. et Eibety. Rationale for using aromatase inhibitors to manage benign prostatic hyperplasia. Experimental studies J. Androl. 12: 356, 1991.
50 LOCAL TESTICULAR CONTROL MECHANISM ENABLED THE TOTAL NUMBER OF SERTOLI CELLS FOUND IN AN INTACT RAT TO BE EXCEEDED. Larry Johnson, Kevin L. Kunde, Genevieve E. Keillor, and Angela J. Kaschmitter. Dept. of Vet. Anatomy & Public Hth., Texas Veterinary Medical Center, Texas A&M University, College Station, TX.

If the total number of Sertoli cells in a rat is to be augmented by intervention, the number of Sertoli cells achievable in an intact rat (46.4 ± 1.0 x 10^6) must be exceeded. Furthermore, if the testis itself contributes locally to the regulation of total Sertoli cell number, transplantation of more than two testes should augment the total number of Sertoli cells achievable per rat. Four, six, and eight neonatal testicular grafts were transplanted into young, adult, andcastrated, Fischer rats. At 20 days posttransplantation, the hosts were terminated, and the testicular grafts were recovered, fixed, and embedded in Epon. Toluidine blue stained 0.5 mm sections were evaluated by stereology to determine the volume density of Sertoli cell nuclei. Unstained 20 µm sections were observed by Nomarski optics to determine the height and width of individual Sertoli cell nuclei to calculate a correct nuclear volume. When four, six, and eight testes were transplanted into five, four, and three rats respectively, there was an increase (p<0.05) in the total number of Sertoli cells (36.0 ± 2.7, 61.6 ± 2.0, and 85.9 ± 2.4). Hence, rats with six or eight transplanted testis had a total number of Sertoli cells/rat that exceeded the number in intact rats. However on a per testis basis, there was no change (p>0.05) in the number (10^6) of Sertoli cells per testis (10.2 ± 6.9, 11.2 ± 2.8, and 11.6 ± 2.8) or in testicular parenchymal weight per testis (10.7 ± 7.0, 12.1 ± 4.4, and 13.3 ± 5.2 mg). Since the total Sertoli cell number can be increased proportionally by increasing the number of testes transplanted, a component of regulation of Sertoli cell number and testicular size is at the level of the testis. In conclusion, the total number of Sertoli cells is amenable to experimental intervention, and a local testicular controlling component of total Sertoli cell number must be involved. NIH RO1 AG10939-10

51 EFFECTS OF EXERCISE ON TESTOSTERONE AND NITRIC OXIDE PRODUCTION IN THE RAT TESTIS. V.J. Meskauskas*. F.S. Hannam*, J.S. Volek*, B.C. Nind*, W.J. Kratzer*, D. Weinstock* and D.R. Desver Departments of Dairy and Animal Sciences and Veterinary Science and the Center for Sports Medicine, The Pennsylvania State University, University Park, PA

Effects of exercise on testosterone secretion varies among species and with the type of exercise program. In rats inhibition of nitric oxide production results in a marked increase in testosterone production. The objectives of this study were to determine: a) if chronic exercise would increase production of testosterone, b) if changes in testosterone secretion could be correlated with unstimulated nitric oxide production, and c) the location of the nitric oxide synthase (NOS) within the rat testis. Thirty-six rats were used for this study and assigned to one of the following treatment groups: a) control - not exercised and killed at rest; b) a single exercise session and killed immediately after (SES); c) a chronic seven-week exercise program and killed 3 days after the last training session (CER); and d) same training program as in (c), but rats were killed immediately after the last training session (CEE). The exercise program used a ladder-climbing paradigm where rats were carrying increased weight up a ladder over the seven-week training period. Rats were exercised three times per week and each period of exercise consisted of 10 climbs with a 2 minute rest period between each two climbs. Body weight did not change during the study and averaged 498 ± 6 g at the end. On the first day of the exercise program the average weight carried was 52.7 ± 6.0 g and this increased to 530 ± 31.2 g at the end of the training period. Leydig cells were killed, and nitric oxide synthase was collected from the other. Plasma concentrations of corticosterone were elevated in rats in the SES and CEE groups when compared to the control and CER groups (p<0.05). The content of TF testosterone lost among the SES and CEE groups (interaction p<0.05). Testicular nitrate content followed the same pattern as TF testosterone, the correlation coefficient between TF testosterone and TF nitrate was 0.781 (p<0.0001). Concentrations of plasma testosterone were also correlated with TF nitrate (r = 0.709, p<0.0001). Leydig cells stained intensely for iNOS. Based on these data we conclude that testicular secretion of testosterone is inhibited in the beginning of an exercise program, likely due to inhibitory effects of glucocorticoids. However, during the course of the seven-week training program the activity of nitric oxide synthase is increased in the rat testis. These effects may be due to altered testicular blood flow and/or direct paracrine action of NO on Leydig cells.
53 **STAGE-SPECIFIC LOSS OF GERM CELLS IN THE RAT AFTER A SINGLE EXPOSURE TO HEAT OCCURS BY APOPTOSIS**


Short term exposure of the testis to heat causes degeneration of germ cells but has not been distinguished necrosis from apoptosis. We have previously demonstrated that stage-specific loss of germ cells after hormonal deprivation occurs exclusively by apoptosis. The objectives of this study were to establish whether heat also causes damage to the testis by apoptosis, if so to document its stage-specific kinetics. Testes of adult male rats (5 rats per group) were immersed in a water bath at room temperature 22°C (control) or at 43°C for 15 min (heat treated groups). Animals were sacrificed on day 1, 2, and 9 after heat exposure. Apoptosis was characterized by a modified in situ procedure which specifically detects apoptotic germ cells in the testis and quantitated as number of apoptotic germ cell per Sertoli cell (apoptotic index, AI). Germ cell apoptosis was markedly increased (p<0.05) on day 1 and 2 after exposure to heat but not on day 9 when compared to control. On day 9, almost all apoptotic cells disappeared and an active proliferation process was noted by presence of increasing number of preproetope and leptotene spermatocytes. On day 1, the mean AI was highest at stages I-V, IX-XI, XII-XIV (3.4 to 5.9), and lowest at stages V-VI (0.34) whereas stages VII-VIII (2.1) were intermediate. On day 2, AI remained highest between stages I-V, IX-XI, XII-XIV (3.1 to 5.5), but the lowest AI occurred at stages VII-VIII (1.1). The increase in AI at stages V-VI (2.7) at day 2 may reflect progression of apoptotic cells from previous stages. The results indicate that 1) exposure of the adult rat testis to heat results in the stage-specific loss of germ cells via apoptosis, 2) the early and late stages of spermatogenesis are most sensitive to the effects of heat on apoptosis. The mid stages V-VI and VII-VIII (hormone response stages) are relatively protected from programmed cell death. We conclude that transient exposure to heat damages the tests by stage specific activation of apoptosis.

55 **TPX-1 GENE IS SPECIFICALLY TRANSCRIPTED BY NORMAL HUMAN GERM CELLS DURING THE LATE STAGE OF SPERMATOGENESIS.**

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**Background:** TPX-1 gene was found to map adjacent to HLA on chromosome 6 and have 17.8% nucleotide 70% amino acid sequence similarity with mouse Tpx-1 gene (Kashara et al. Genomics 5:357, 1990). TPX-1 gene is considered to be the human counterpart of mouse Tpx-1 gene. Mouse Tpx-1 is a testis-specific gene and transcribed by mouse haploid male germ cells. However, the specificity and the function of Human TPX-1 gene remained unknown.

**Objectives:** To study the activation site of the TPX-1 gene and to investigate the expression of this gene product in various tissue samples.

**Methods:** Total cellular RNA from tissue samples was extracted by the guanidinium-isothiocyanate method. After transferring RNA to a Gene Screen Plus (NEM, Boston, MA), hybridization was made to the 32P-labeled nick-translated TPX-1 probe. Approximate sizes of the hybridizing RNA were estimated using mouse ribosomal RNA as molecular size marker.

**Results:** Here we show that the messenger RNA derived from this gene (1.4 and 2.0kb) is specifically and abundantly transcribed in normal adult human testis but not in epididymis, prostate, or ovary. Moreover, we could not detect this gene expression in a testis biopsy sample of infertile patient with maturation arrest, an infant undescended testis biopsy sample, or various testicular tumors. These results suggest Tpx-1 gene is a testis-specific gene and transcribed by normal human male germ cells during the late stage of spermatogenesis, raising the possibility that TPX-1 may play an important role in the human spermatogenesis and thus fertilization.

56 **BIOCHEMICAL CHARACTERIZATION OF SERTOLI CELL (SC) MEMBRANE PROTEINS TO BE INVOLVED IN SPECIALIZED JUNCTIONAL COMPLEX (JC) FORMATION.**

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Throughout spermatogenesis, primitive germ cells traverse from the basal lamina to the adluminal compartment of the seminiferous epithelium where mature spermatzoa are released into the tubular lumen. It is conceivable that inter-Sertoli and Sertoli-germ cell specialized junctions are continuously disrupted and regenerated. Thus, JC component proteins are expected to turnover rapidly. This study sought to identify SC membrane proteins that are likely to be involved in these events utilizing [3H]Met incorporation. Pure SC isolated from immature rats were cultured atigh cell density (0.7 x 106 cells/cm2) with [3H]Met on Matrigel-coated dishes in Ham's F12/DMEM in order to allow the formation of specialized junctions. Thereafter, SC were processed for membrane extraction by cell lysis under hypotonic conditions, homogenization and detergent solubilization. The solubilized [3H]-labeled proteins were fractionated by sequential C8 and C4 reversed-phase HPLC and SDS-PAGE. Actively synthesized proteins of 45, 38, 28, 18.5, 18, 17.5 and 13 kDa proteins were further purified to apparent homogeneity by successive HPLCs. Partial N-terminal and internal amino acid sequence analysis revealed that some of these proteins are indeed unique. Similar protein patterns were seen using testicular membrane extracts except that the 45, 38, 18.5, and 18 kDa proteins were more prominent in the SC membrane extract. When the same amount of protein obtained from SC membrane extract derived from cells cultured at low cell density (5 x 104 cells/cm2) in the absence of specialized junctions was examined, the abundance of the 45, 38, and 18 kDa proteins was significantly reduced suggesting that these proteins may be related to JC formation. In summary, a unique set of proteins are identified in the SC membrane when JC are formed in vitro suggesting that these proteins may be important for JC formation and are novel markers in studying cell-cell interactions in the testis.

57 **SERTOLI CELL CYTOSKELETAL AND RELATED PROTEINS, AND α2-MACROGLOBULIN, ARE NOT INVOLVED IN INHIBITED SPERMATION (I.S.).**

R.N. Wine* and R.E. Chapin. Reproductive Toxicology Group, NIEHS, RTP, NC

A common feature of the testicular response to many toxicants is inhibited sperm release. We used immunohistochemistry to evaluate I) cytoskeletal proteins (tubulin, vimentin, actin, vinculin, and α-actinin) because several Sertoli cell cytoskeletal proteins are closely apposed to germ cells, and have been related to movement of germ cells within the seminiferous epithelium, 2) β3 integrin, a member of a family of intercellular adhesion molecules, and 3) the protease inhibitor α2-macroglobulin (α2-M), found to be not present specifically at spermatogenesis. SD rats, 3/group, were treated with doses of compounds known to produce I.S. (boric acid, dibromoacetic acid, 2.5-hexahydroxy, or tri-cresyl phosphate). Testes were snap frozen or fixed in paraformaldehyde prior to cryostat sectioning, and staining. Antibodies were obtained commercially, except α2-M antibody, kindly provided by Yan Cheng (Pop a. Council, NY). Bound antibody was visualized with Zymed kits. The distribution of staining was the same for both control and toxicant-treated testses. Tubulin was prominent in the apical Sertoli process, was not associated with normal step 19 spermatids, and was not localized adjacent to aberrantly-retained spermatids. Similarly, actin was prominent around the spermatid head but was not near step 19 spermatids at normal spermatiation, and was not localized near retained spermatids. Vimentin, vinculin, and α-actinin, clearly visible around Sertoli nuclei and extending luminally, were never associated with retained spermatids. α2-M was distributed as previously described (Zhu et al., J. Androl. 15:757, 1994), and showed no alteration with I.S. β3 integrin staining was found in late spermatid cytoplasm and residual bodies, but was not near the heads of retained spermatids. Proteins comprising, and related to, the Sertoli cell cytoskeleton appear not to mediate I.S.; current efforts focus on changes in adynamal proteases.
57 5-azaCytidine treatment initiated during meiotic development of male germ cells results in abnormal embryonic development. In previous work, administration to male rats of 5-azaCytidine (5-aza), a cytidine analogue that leads to decreases in DNA methylation, resulted in abnormal embryonic development when germ cells were exposed during mitotic, meiotic and post-meiotic development but not if they were only exposed post-meiotically. To determine whether germ cell exposure initiated during meiotic development could also alter sperm function, male Sprague-Dawley rats (n=6/group) were treated with saline or 2.5.5 L L and 4.0(H) mg/kg of 5-aza, 3 times a week for 6 weeks. To assess progeny outcome, males were mated with normal females and cesarean sections performed at 20 days of gestation. Maternal 5-aza treatment resulted in a dose-related increase in preimplantation loss (saline-5.0%, 5-azaL-9.5%, 5-azaM-24.2%) with no increase in post-implantation loss or fetal abnormalities. Embryos were also examined at 17 hours (pronuclei present) and 24 hours (2-cell stage) of age to determine whether the early embryonic death was due to lack of fertilization or embryo death. At 17 hours, embryos in all groups showed a pronucleus and sperm tail, indicating that fertilization had occurred. In contrast, on day 2 of gestation, mating with the 5-aza treated males resulted in an increase in the number of morphologically abnormal embryos (saline-7.9%, 5-aza-16.4%, 5-aza-31.3%). The results provide evidence that the preimplantation loss seen at 20 days results from death of embryos early in their development, at around the 2-cell stage. We suggest that 5-aza leads to decreases in DNA methylation in meiotic germ cells that subsequently affect embryo development. (Supported by MRC)

58 Expression of cyclin H and cyclin-dependent kinase 7 in the mouse testis J.T. McGeary and S.E. Reamik, Dept. of Cell Biol. & Biochem., Texas Tech University Health Sciences Center, Lubbock, TX 793-30.

Cyclin/Cyclin-dependent kinase (Cdk) complexes are key regulators of the cell cycle. We have previously shown that cyclin A1 and cyclin A2 and their Cdk partners, Cdc2 and Cdk2, are differentially expressed in the mouse testis during spermatogenesis. Recently, cyclin A/Cdk2 complexes in cell lines were shown to be activated by another cyclin/Cdk complex, cyclin H/Cdk7 or CAK (Cdk Activating Kinase). To begin to test the hypothesis that CAK regulates the cyclin A-associated kinases during spermatogenesis, we have begun to examine the expression of cyclin H and Cdk7 in the mouse testis. Analysis of RNA in several adult tissues (heart, kidney, liver, lungs, ovary, and testis) revealed two components, the first being a low K. species that may function in both mitotic and meiotic cell cycles. In situ hybridization analyses to determine somatic or germ cell origin and the cellular localization of cyclin H and Cdk7 are ongoing. These studies will provide further insight on the activation and regulation of the cell cycle proteins that control meiosis. (Supported in part by funds from the South Plains Foundation (SER) and by a grant from the Howard Hughes Medical Institute through the Undergraduate Biological Sciences Education Program (ITM)).

59 Effect of thyroid hormone on testicular development and mRNA levels of thyroid hormone receptors in the testes of immature and adult rats J.N. Ran, J-Y. Liang*, J.F. Wilber* and P. Feng*, Division of Endocrinology, University of Maryland School of Medicine, Baltimore, MD 21201.

Thyroid hormone (T3) together with its receptors is critical for normal growth, development and metabolism in mammals. To explore the effects of T3 on the regulation of testicular function and its own receptors, we investigated testicular development, and mRNA levels of TR-α and β in the hyper- and hypothyroid testes of immature and adult rats by semi-quantitative RT-PCR. Sprague-Dawley male rats were treated either with T3 or PTU from day 1 to day 21. One half of each group of rats, including control, T3 and PTU treatment, were sacrificed at day 21. The remaining half of each group was allowed to recover until day 50. Significant decreases of body and testes weight were observed in both hyper- and hypothyroid rats of 21- and 50-day-old compared to controls. Higher serum T3 levels found in hyperthyroid rats of 21-day-old and lower levels in hypothyroid were all restored to normal in adulthood. Histological examination revealed reduced size of the tubules and less germ cells in the 21-day-old hypothyroid testis. At 21 days of age, lower testicular TR-α mRNA levels were detected in the hypothyroid group (0.38±0.11, P<0.05), and higher levels were observed in hypothyroid rats (0.98±0.04; P<0.05) compared to the controls (0.79±0.04). No significant changes were detected in TR-β mRNA levels between experimental groups. At 50 days of age, neither testicular TR-α nor TR-β mRNA levels were altered significantly. Conclusions: Our results demonstrated that prepuberal hyper- and/or hypothyroidism can directly influence 1) testicular development 2) mRNA levels of TR-α but not of TR-β in the immature testis, and 3) the altered levels of testicular TR-α mRNA could be restored to normal at adulthood.


Leydig cells are susceptible to the direct glucocorticoid-mediated inhibition of testosterone biosynthesis. This inhibition can be countered by 11β-hydroxysteroid dehydrogenase (11β-HSD), which oxidatively inactivates glucocorticoids. Of the two isoforms of 11β-HSD that have been identified, type I is an NAD(P)-dependent oxidoreductase with reductive activity predominating. In contrast, type II is NAD-dependent and acts exclusively as an oxidase. The identity of the 11β-HSD isoform in Leydig cells is uncertain, since the protein in this cell is recognized by an anti-type I 11β-HSD antibody but the activity is primarily oxidative, more closely resembling type II. The goal of the present study was to determine whether the kinetic properties of 11β-HSD in Leydig cells are consistent with type I, type II or neither isoform. Leydig cells were purified from male Sprague Dawley rats (250 g), and 11β-HSD oxidative and reductive activities, types I and II mRNA levels, and type I protein were evaluated in Leydig cells. Leydig cell 11β-HSD had bidirectional catalytic activity that was NADP(H)-dependent. This corroborated the hypothesis that type I 11β-HSD is present in rat Leydig cells. However, unlike the type I 11β-HSD in liver parenchymal cells that is primarily reductive, oxidative predominates in Leydig cells. Moreover, analysis of kinetics revealed two components, the first being a low K. NADP-dependent oxidative activity with a K. of 41.5 ± 9.3 nM and V. of 7.1 ± 1.2 pmol/min/106 cells. The second component consisted of high K. oxidoreductase activities that were consistent with type I NADP-dependent oxidative activity with a K. of 5.87 ± 0.46 μM and V. of 419 ± 17 pmol/min/106 cells, and NADP-dependent reductive activity with a K. of 0.492 ± 0.031 μM and V. of 17 ± 6 pmol/min/106 cells. The RT-PCR and immunoblotting data confirmed that type I 11β-HSD mRNA and protein are present in Leydig cells, whereas type II mRNA was undetectable. Immunoblot of Leydig cell cytosol showed higher molecular mass proteins at 40 and 68 kDa, in addition to the 34 kDa species that is typical of type I protein. We conclude that rat Leydig cells possess a low K. oxidative activity and therefore may contain another kinetic isoform of 11β-HSD in addition to the previously characterized type I. Supported by CONRAD (R-S.G.), the Rockefeller Foundation (H.-B.G.) and NIH grant HD-33000 (M.P.H.)
were not affected by any of the treatments. In contrast, significant increases in DNA weight of the prolactin (250 ng/kg/day) and estradiol (0.4 µg/kg/day) silastic capsules at 2 years of age (tr1), estradiol at 2 years of age and immediately implanted with testosterone and estradiol silastic capsules as in (tr3). The implants in all groups were replaced at nearly intervals every 6 years of age, at which time the prostates were removed and dissected. For all specimens, a transverse segment was taken from the mid-apical portion including central and peripheral zones. Part of the tissue was immediately placed in protease inhibitor buffer and homogenized, the remainder snap frozen and cryostat sectioned. The homogenate was processed for Western blot analysis using a well-characterized rabbit anti-neuronal NOS (nNOS) antibody. Cryostat sections were processed using routine histopathological techniques. RESULTS: In tr1 (n=7), complex BPH with a prominent glandular component was observed in all prostates. nNOS immunoreactivity was absent in all specimens. In tr2 (n=10), moderate to severe atrophy was observed in 7 prostates, normal histology with small areas of hyperplasia, and mild BPH in prostate. Five of the 7 atrophy exhibited nNOS expression. In tr3 (n=9), moderate to severe glandular atrophy was observed in 7 prostates, normal histology with small areas of hyperplasia in 1, and moderate BPH in 1. Six of the 7 showing atrophy exhibited nNOS expression. CONCLUSIONS: These data describing the absence of nNOS expression in BPH tissue and, conversely, its distinct expression in atrophic prostatic tissue following hormone alterations (79%) suggest that NO is inversely related to growth and development of the canine prostate and may be androgen-regulated. Whether nNOS deficiency contributes to the pathogenesis of BPH or occurs as a consequence of this disease process remains to be determined. Work supported by NIH grant DK-19300.

62. SYNERGISM OF ESTRODIOL AND TESTOSTERONE ON BROWN NORWAY RAT PROSTATE GROWTH. P.P. Bavereri, R.R. Zirkin and T.R. Brown, Division of Reproductive Biology, Johns Hopkins University, Baltimore, MD.

Physiological and pharmacologic doses of exogenous testosterone cause age- and lobe-specific overgrowth in Brown Norway rats. Hyper trophy occurs in the ventral (VP), dorsal (DP) and lateral (LP) prostatic lobes of young and old rats, whereas DNA contents, a measure of cell number, increase in the DP and LP, as a function of testosterone dose and age, but not in the VP. We subsequently discovered that age- and lobe-specific overgrowth of the prostate occurs spontaneously in the absence of exogenous hormone treatment. Western blot analyses revealed a decrease in androgen receptor protein level in all three lobes of old rats. By contrast, a lobe-specific (DP and LP) increase in estrogen receptor protein level was observed with age. These results prompted us to investigate the effects of estrogen on the age- and lobe-specific overgrowth of the Brown Norway rat prostate. Young (4 months) and old (22 months) rats were implanted with 2.5 cm testosterone (T), 0.1 cm estradiol (E), or a combination of T+E filled silastic capsules for 10 days. The wet weight of VP increased slightly (+10%), but not significantly, in young and old rats, respectively. Estradiol increased wet weight of the DP and LP (64% and 55%, respectively), whereas T caused a significant increase (+25%) in LP weight at both ages. Similarly, in the DP and LP, T treatment increased wet weight by (+55%), but not significantly, in young and old rats, whereas E treatment caused significant decreases (50%) at both ages. Strikingly, treatment with T+E significantly increased wet weights of the DP and LP by 64% and 75%, respectively, in young rats and by 67% and 54%, respectively, in old rats. DNA content did not change significantly in the VP of young or old rats after treatment with T alone or with T+E, but E alone caused significant decreases of 75% and 55% in young and old rats, respectively. In young rats, the DNA contents of the DP and LP were not affected by any of the treatments. In contrast, significant increases in DNA content were observed in both DP and LP in old rats following T+E treatment, but not with E or T separately. Histologically, ducts in the DP and LP appeared more highly infolded with crowding of epithelial cells following T+E treatment. In summary, E synergized with T in all three lobes of the rat prostate, but increases in DNA content occurred only in the DP and LP of old rats. These results suggest that E can potentiate DNA synthesis, and thus may have a role in the development of lobe-specific spontaneous overgrowth of the Brown Norway rat prostate. (Supported by AG08321)


Gamma-glutamyl transpeptidase (GGT) is an enzyme believed to play a role in the patency of the male reproductive system. In particular, the expression of GGT mRNA-IV in the epididymal initial segment is dependent upon the presence of testicular factors. Efferent duct ligation (EDL), which prevents testicular flow to the epididymis, results in a 50% decrease in the expression of GGT mRNA-IV in the initial segment after 12 hours. The objective of this study was to test the hypothesis that the decreased expression of GGT mRNA-IV in the initial segment following the removal of testicular factors is due to a decrease in transcription rate and/or an increased rate of decay. Changes in transcription rate were evaluated by examining changes in the amount of newly transcribed mRNA produced at a given time. The expression level of an intron present in the 3' untranslated region specific to GGT mRNA-IV was assayed using RNASse protection analysis and served as a measure of newly transcribed mRNA-IV. Results of this analysis revealed that the transcription rate of GGT mRNA-IV in the initial segment fell by ~70% following EDL. Decay of the stability of an mRNA were determined by evaluating mRNA levels following incubation of tissue in a transcription inhibitor for varying amounts of time. These stability studies showed that immediate removal of testicular factors from the initial segment induced a rapid initial decay of GGT mRNA-IV (t1/2 = 0.86 hours; 0-1 hours) which slowed at later times (t1/2 = 10.5 hours; 1-24 hours) to a decay rate paralleling mRNAs-III. Thus, the decreased expression level of GGT mRNA-IV in the initial segment following the removal of testicular factors is a function of both a decreased transcription rate and an increased rate of decay.

Supported by N.I.H. grant HD29797 (BTH), N.I.H. P30-HD28934, and training grant N.I.H. T32-DK07642 (DBR).


Although estrogen receptors have been localized in the epithelium and connective tissue of ductular efferentes (efferent ductules), the functional role of estrogen in the male reproductive system is unknown. The recent establishment of the estrogen receptor knock out mouse (ERKO) has allowed us to investigate the function of the ductular efferentes. EXPERIMENT 1. Adult +/- and ERKO mice were anaesthetized and the initial segment epididymis on one side was occluded by catherization and the animals were allowed to recover. At 48 h post occlusion the testes were removed and weighed and the difference in weight between the occluded and contralateral side was determined. The data show that the occluded ERKO mouse testis had a significantly greater increase in weight at 48 h than was found in the +/- animal. Thus, the ERKO mouse efferent ductules did not reabsorb luminal fluids at the same rate as did normal ductules. EXPERIMENT 2. Individual efferent ductules were dissected into 0.5-mm segments, incubated in radiolabeled culture medium with tritiated thymidine and tritiated thymidine (3H1) and 3H-estradiol at 34°C for 60 hours. After 60 hours, the segments were ligated at one end with silk suture to retard the 3H label. A 0.5-mm transverse segment was excised from the midpoint of the ligated segment and the luminal fluid was aspirated and counted for radioactivity. The luminal diameter of the proximal and distal segments were determined. The data show that the occluded ERKO mouse testis had a significantly greater increase in weight at 48 h than was found in the +/- animal. Thus, the ERKO mouse efferecent ductules did not reabsorb luminal fluids at the same rate as did normal ductules. The data show that the occluded ERKO mouse testis had a significantly greater increase in weight at 48 h than was found in the +/- animal.
65 EFFECT OF TESTICULAR BIOPSY AND TRIETHYLENEMELAMINE ON TESTIS FUNCTION AND SEMEN QUALITY IN THE DOG R.H. Foote, Department of Animal Science, Cornell University, Ithaca, NY.

Semen was collected 3X per week for 5 weeks from 27 sexually mature kennel-reared Beagle dogs. After 5 weeks 20 of the dogs with good libido and trained for semen collection were divided into four groups of five dogs each. Semen collection was continued 3X per week (Monday, Wednesday and Friday) for 20 weeks. Group I was a control with no treatment until unilaterally castrated at week 16. Group II had three testicular biopsies on one testis on weeks 1, 5 and 9, with this testis-epididymis removed on week 16. Groups III and IV received, respectively, 0.2 mg/kg and 0.4 mg/kg of body weight, of triethylentenamine (TEM) intravenously at the outset of the 20-week test period. These dogs also had testicular biopsies on weeks 1, 5 and 9 and a unilateral castration on week 16. All dogs were castrated on week 20. The average sperm output per week during the first four experimental weeks in Groups I and II was 760 and 876 million, respectively, and during the last four weeks before unilateral castration (weeks 13-16) was 685 and 811 million, respectively. Thus, the controls decreased 9.9% and the biopsied group 7.4%, indicating a possible seasonal effect, but no detectable biopsy effect. Unilateral castration reduced sperm output by 47 and 46% in groups I and II, respectively. In the TEM-treated groups III and IV, weekly sperm output during weeks 1-4 averaged 933 and 1094 million, respectively. By weeks 13-16 corresponding average weekly sperm output was markedly depressed to 375 and 126 million. The percentage of motile sperm was not affected.


Previous sperm cryopreservation studies in lefids reveal loss in sperm motility and extensive acrosomal damage post-thaw. To evaluate factors that affect sperm structure and viability during freezing, we analyzed the effects of cooling on sperm from normospermic (N; >60% normal sperm/ejaculate, n=3 males) and teratospermic (T; <40% normal sperm/ejaculate, n=3 males) domestic cats. Ejaculates were divided into either raw or washed (Ham’s F10+5% fetal calf serum; FCS) aliquots. Washed pellets were resuspended in: 1) Ham’s F10+5% FCS; 2) PDPF (20% egg yolk, 11% lactose); and 3) Refrigeration Medium/Test Yolk Buffer (OTYB). Sperm aliquots were maintained at 25°C (control) or exposed to: 1) 0°C for 10 min; and 2) 4°C for 30 min. Following warming to 37°C, samples were evaluated at 0, 5, 60, and 240 min for sperm motility and percentage of normal acrosomes (using FITC-PNA. In all treatments, sperm motility was not affected (P=0.05) by cooling. However, % intact acrosomes was reduced (P=0.05) in raw, Ham’s F10 and PDPF aliquots in both cat populations following exposure to 0°C (N, 39-63%; T, 19-34%) or 4°C (N, 54-88%; T, 23-39%) compared to 25°C (N, 75-84%; T, 71-73%). In contrast, OTYB maintained (P=0.05) ejaculate integrity in normospermic ejaculates exposed to 4°C (71%), but provided less (P<0.01) protection at 0°C (87%) compared to 25°C (81%). Furthermore, % intact acrosomes in OTYB was reduced (P=0.01) in teratospermic ejaculates at both 0°C (24%) and 4°C (37%). These results indicate that: 1) cooling induces acrosomal damage in domestic cat sperm without altering sperm motility; 2) PDPF fails to protect cat sperm acrosomes during cooling, while OTYB is only protective for normospermic ejaculates cooled to 4°C; and 3) teratospermic ejaculates are highly susceptible to acrosomal damage during cooling compared to normospermic ejaculates. (NH grant #23853; Smithsonian Institution Scholarly Studies Program).


on testis is fun ction and semen quality in the dog R.H. Foote, Department of Animal Science, Cornell University, Ithaca, NY.

6.7 Many zoos in North America pair cheetahs for breeding; in January and July, in the northern and southern hemispheres respectively. A review of birth dates reported in the International Cheetah Studbook reveals captive births in April and October in the northern hemisphere and January and July in the southern hemisphere. The increase in births seen in the spring and fall may be the result of winter pairings, or could reflect physiological responses to environmental cues. It is of interest to determine the role semen production may play in annual reproductive rhythms. Semen was collected weekly by artificial vagina from three male cheetahs over a three year period to determine the effects of season on semen quality. Males #1 and #2 (6 and 5 years of age respectively) were hand-reared individually with no opportunity to breed. Males #3 (4 years of age) was a mother-reared proven breeder. Semen analysis included volume (V, ml), concentration (C, 1010 cells/ml), motility (MOT) and speed of progression (SOP, scale of 1 to 5, 3 being fastest), and percent normal morphology (%N). Motility scores (MS) were calculated as MS = V x SOP². To facilitate comparison of males and seasons, semen quality indices (SQI) were calculated as (V x C x %N)/100. Means, ranges and number of ejaculates for each measured semen parameter for all males during the study were: V, 1168-11500 ml; C, 374-713 million; %N, 36.0-96.2; n, 20-46; M, 36.6-91.1; SOP, 120.0-97.9, n=173. Within males, volume of ejaculate was highest for each male during the summer, but was significant only for Males #1 and #2 (p<0.04). Between males, Male #3 had a higher (p<0.05) mean volume during the winter season compared to both Males #1 and #2. Male #3 exhibited greater (p<0.05) sperm concentration during the summer and fall compared to both Males #1 and #2. The percentage of morphologically normal sperm did not differ between males during the study. However, Male #3 had normal sperm (40%) during the winter, but not at any other time. Male #3 had a higher (p<0.05) mean sperm motile during the summer than Male #1. When all parameters were factored into the SQI, there were no significant differences between males during any of the four seasons, although there was a tendency for each male to have higher SQI in the winter than in other seasons.

<table>
<thead>
<tr>
<th>Male</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>within</th>
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</thead>
<tbody>
<tr>
<td>#1</td>
<td>1202 ± 285</td>
<td>972 ± 249</td>
<td>664 ± 228</td>
<td>982 ± 78</td>
<td>n/a</td>
</tr>
<tr>
<td>#2</td>
<td>1485 ± 707</td>
<td>1217 ± 85</td>
<td>873 ± 169</td>
<td>923 ± 173</td>
<td>n/a</td>
</tr>
<tr>
<td>#3</td>
<td>1603 ± 41.9</td>
<td>1213 ± 274</td>
<td>1743 ± 260</td>
<td>1307 ± 36.9</td>
<td>n/a</td>
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<td>between</td>
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These data indicate that there are no seasonal differences in sperm quality in captive male cheetahs.

68 EJACULATORY CHARACTERISTICS OF THE BLACK-HANDED SNAKE, PYTHON BLACK WALLOWER ANOUA BLACK WALLOWER J.A. Long, N. Lemberski* and AH Shoemaker* Conservation Research Program, Riverbanks Zoological Park and Botanical Garden, Columbia, SC.

Considering the large number of primate species, basic knowledge about reproductive physiology is available for only a disproportionate few. In light of the current biodiversity crisis, it is essential to begin accumulating data for little-studied species. Objectives here were to examine seminal traits of the black-handed spider (Ateles geoffroyi), black howler (Alouatta caraya) and the endangered Diana (Cercopithecus diana) monkey. Ejaculates were collected from anesthetized males (2 ejaculates/male) using either a 1.3 cm (spider monkey) or 1.6 cm (howler and Diana monkey) rectal probe. Electrical stimuli were delivered in serially-increasing increments (2-6 volts; 10 stimuli/voltage) until semen was obtained. Following an initial assessment of sperm motility, ejaculates were diluted 1:1 with Ham’s F10 (± 5% fetal bovine serum). Ejaculate volume varied extensively among the 3 species (spider monkey, 3 ml; howler monkey, 0.12 ml; Diana monkey, 0.02 ml). Howler monkey ejaculates exhibited no coagulum; however, Diana monkey ejaculates required 30 min (37°C) to complete liquefaction. In contrast, spider monkey ejaculates coagulated within 2 min of collection and did not dissolve completely, even after a 2 h incubation (37°C) in Ham’s F10. Although the coagulum remained intact, 3 x10⁶ sperm/ml were present in medium asparaged after 30 min of incubation. Fewer sperm (0.4 - 1 x10⁶ sperm/ml) were recovered after 1, 1.5 and 2 h of incubation. Sperm concentrations in howler and Diana monkey ejaculates were 0.8 and 10 x10⁶ sperm/ml, respectively. High numbers of morphologically normal sperm were observed in howler (52.4%) and spider (41.8%) monkey ejaculates; whereas Diana monkey ejaculates contained few structurally normal sperm (15.5%). Diana monkey sperm anomalies included flagellar (36.2%) and multiple (cerebral + flagellar, 22.4%) defects. Initial sperm motility was poor (20%) in Diana monkey ejaculates compared to howler (80%) and spider (80%) monkey ejaculates. Collectively, these data demonstrate the unique ejaculate characteristics of these primate species and will help to establish interspecies, including ejaculate traits, seasonal variations and reproductive soundness.

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An increased role of neural bodies and ovarian release of HCG on ovulation resulted in increased gonadotrophin levels in the serum monitored through the administration of recombinant FSH (rFSH; Ares Serono: 10 IU/kg) for six days followed by r-hCG plus r-MHC (Ares Serono: 10 IU each 12 h) for one to three days for three stimulation cycles (C1, C2, C3). r-MHC (Ares Serono) 1000 IU IM was given the day following the appearance of follicles greater than 10 mm on ultrasound. Surgery to aspirate follicles occurred 10 to 15 hours later, with both monkeys, during C1 and C2. E2 peaks were similar at r-MHC administration, but in both cases had lower E2 peaks and spontaneous drops of E2 levels after the E2 drop.

Although recombinant gonadotrophins did not successfully produce a third stimulation, but the production of IVF stimulations is a significant event with these highly endangered primates.

### 71 INCREASED FERTILITY IN TURKIES RESULTING FROM SEMEN DONOR SELECTION A.M. Donohue*, Germplasm and Game Physiology Laboratory, ARS, USDA, Beltsville, MD 20705 and D.P. Froman* Department of Animal Sciences, Oregon State University, Corvallis, OR, 97331

Commercial turkey production depends exclusively upon artificial insemination of pooled semen. The objective of this study was to pre-screen individual males using an objective motility assay. Semen was then pooled by motility ranking and fertility evaluated. The motility assay differs from conventional motility assessment for poultry in that the sperm must overcome a physical barrier to forward movement. Semen from each ejaculate (n=75 toms) was diluted to 1x10^6 sperm/ml in pre-warmed TES based motility buffer and placed over 3 ml of a 3% Accudenz solution (Accurate Chemical, Westbury, NY) in a polyurethane cuvette at 41°C. After a 5 min incubation the cuvette was placed in a densimeter and % motility recorded after 1 min. Semen from toms ranked in the highest and lowest 10% after 3 screenings were pooled by group and used to inseminate hens weekly (n=20 hens/group) for 10 weeks. Fertility from hens inseminated with semen from the HIGH MOTILE males averaged 95.8±1.3% over the 10 weeks and was higher than fertility from the LOW MOTILE tom group 90±2.2% (P<0.05). Hatchability of fertilized eggs did not differ between groups. Weekly motility evaluations on semen were consistently higher for the HIGH MOTILE males than the LOW MOTILE males throughout the study. Motility differences between males influenced fertility outcome. By mimicking the hens vagina, this assay may identify males whose sperm have a greater ability to reach the sperm storage tubules and subsequently fertilize oocytes.
In animal agriculture, elimination of males likely to be subfertile is of economic importance. An in vitro sperm-bonding assay utilizing segments of hen's egg membrane (BOR, 50 Suppl 1:128, 1994) was modified to incorporate (1) pre-labeling of extract containing perivitelline membrane protein with AMCA, (2) drying 50 µg protein/well in a microwell plate, (3) incubation of replicate sperm suspensions (buffer control or 0-23-10 x 10^6 sperm/well) for 30 or 60 min at 37°C, (4) washing 3X to remove unbound sperm, (5) drying at 60°C for 18 hr, (6) staining DNA with a YOPRO and protease cocktail, (7) reading fluorescence at 485/530 to quantify DNA and 360/460 to quantify binding-protein, and (8) expression of data as sperm bound per 50 µg protein and % bound. The assay likely is applicable to mammalian sperm as the perivitelline membrane of a hen's egg is similar to the zona pellucida. For bull, horse, human, mouse, rooster or turkey sperm, binding was negligible for sperm rendered immobile by blocking energy transduction or snap freezing, whereas motile fresh or frozen-thawed sperm bound in a dose-responsive manner. % bound sperm was maximal at 0.25-2 x 10^6 sperm/well. Capability of the assay to detect males likely to be subfertile was evaluated with chickens. Data for 2 of 5 studies are presented. [A] Sperm in 3 ejacula from each of 28 roosters of unknown fertility were evaluated by the sperm-bonding assay and applying a rule of 2 out of 3. 7 bad roosters were identified, semen from each was used to inseminate 5 hens (3X 4 x 10^6 10^6 x 10^6 sperm). Fertility of the 7 bad roosters averaged 70% vs 76% for the other 21 males; there was 1 of 7 false positives. [B] 57 roosters of a different genetic line were used in a similar study but with 6-7 hens/male, for the bad quartile fertility was 69% vs 88% for the top quartile. USDA-92-202-073, USDA-95-00178 and USDA-96-03883

**74 TESTICULAR MORPHOLOGY AND GONADOTROPIN RECEPTORS REFLECT AGE-RELATED ALTERATIONS IN FUNCTION**


Our studies have focused on reproductive aging in male Japanese quail. During aging, the male shows a loss in fertility and cessation of sexual behavior, both of which precede measurable changes in plasma levels of gonadotropins or gonadal steroids. Further, in vitro Leydig cell function revealed that these cells retain function in spite of regression of the testes. In these experiments we investigated morphological changes that accompany age-related testicular regression and alterations in the number and response of the gonadotropin (LH and FSH) receptors. Male quail were grouped according to age and sexual activity; in addition, great numbers of young photostimulated males and old testosterone implanted males were studied. Histological analyses showed increased incidence of testicular abnormalities during aging; with a high proportion of regressed tissue, collapsed seminiferous tubules, increased lipofuscin and cysts. Specific binding of LH or FSH as a measure of gonadotropin receptors showed an age-related decline in both gonadotropins. Exogenous testosterone stimulated LH receptor number, but did not affect FSH receptor number. These results confirm age-related deterioration in gonadal condition, which is accompanied by altered morphological and receptor characteristics. Further, the gonadotropin receptors did not consistently respond to gonadal steroids as the bird aged.

**75 VARIABILITY AND POWER CALCULATIONS OF VIABILITY ESTIMATES IN DUTCH BELTED RABBIT SPERM**

MICHAEL J. BREITENBERG*, E.M. SCHOLER and S.D. SIMON*. National Institute for Occupation Safety and Health, Cincinnati, OH.

The use of vital stains for assessing sperm viability is a subjective measure of the number of live cells (not stained) vs dead cells (absorbing stain). It can be difficult to distinguish if the cell has absorbed the dye. Often the distribution of the live/dead ratio is not uniform across the slide. In this study an objective viability method was assessed using a flow cytometer. A Coulter Epics Elite flow cytometer with an air cooled argon laser was used. When cells are mixed with the LIVE/DEAD FERTILITY™ sperm viability kit reagents (Molecular Probes, Eugene, OR) and excited with visible-wavelength light the cells fluoresce. The two components of the kit SYBR 14 and propidium iodide (PI) bind to the DNA of the sperm. SYBR 14 is a membrane-permeant nucleic acid stain and PI is the conventional dead-cell stain. Three different populations can be distinguished on the cytogram, live cells, dead cells and dual stained cells. Semen samples were collected from 70 Dutch Belted rabbits for 5 weeks. The semen was collected by ejaculation with four ejaculates per rabbit, 10 µl of the first ejaculate was diluted with 500 µl of Ham's F-10 and used to measure viability. The average percent of dead cells over the five week period was 17.9%. The intraassay correlation, a measure of relative consistency within a rabbit, is 18.4%. This low value implies that a single measurement on a rabbit is not representative of the rabbit's overall mean level. This measure also has fairly large relative variability, both within rabbits and across rabbits. The within rabbit coefficient of variance (CV), a measure of relative variability for an individual rabbit, equals 35.4%. The total CV, a measure of relative variability across all rabbits, equals 46.9%. In spite of this variability, this measure has good power for detecting a shift in a longitudinal design. For example, an experiment with 15 rabbits, with 5 pre-exposure and 5 post-exposure measurements, there is a 94% chance of detecting an average increase from 18% dead cells pre-exposure to 22% dead cells post-exposure.


Our previous findings provide evidence that inhibin is a major factor controlling FSH secretion in 6-month-old bulls (J Endocrinol, 1993, 15-19). However, the regulation of FSH secretion in infant bulls has not been clarified yet. In the present study, we further investigated the physiological significance of testicular inhibin in the regulation of FSH secretion during the early life of bulls, by 1) immunoneutralization of endogenous inhibin and 2) immunohistochemical detection of testicular inhibin. Animals were given a single i.v. injection of 40 ml inhibin antisera at 7 days of age. The spermatogenetic stages in this study, raised against bovine 32 kDa inhibin in a castrated male goat, recognizes the α-subunit of bovine inhibin. Treatment of control serum caused no significant changes in plasma FSH levels during 48 h post injection at all ages. Concentrations of plasma FSH showed a marked increase (p<0.01) after administration of inhibin antisera at 60 and 120 days, while no significant changes were noted in plasma FSH at 7 and 21 days (animals at 21 days old showed a tendency to rise in FSH after administration). Within the solid seminiferous cords at 7, 21, 60 and 120 days, an intense immunostaining was found using the inhibin α-subunit polyclonal antibodies, while there was no immune reaction in the interstitial tissue. The present results strongly suggest that the negative feedback regulation of FSH secretion by inhibin is not fully functional until 21 days old and is completed by 60 days after birth, although Sertoli cells may acquire the ability to produce inhibin earlier than the establishment of the control of FSH secretion by inhibin.

P-43
Previous studies have shown that sperm recovery by MonoPercoll procedure is significantly better than the twoway technique for male factor patients and it is significantly more. Therefore, the purpose of this study was to compare the effect of MonoPercoll procedure and the two-layer Percoll on sperm characteristics of normozoospermic men. Sperm specimens from 10 normal donors were allowed to liquefy prior to processing. Sperm specimens were divided into two equal aliquots. One aliquot was processed by layering 1 mL of 80% Percoll (MonoPercoll), and the other on a two-layer Percoll (Permash) gradient (lower phase 90% and 47% upper phase). Both aliquots were centrifuged at 1600 rpm for 20 min at room temperature. The supernatant was removed and pellet resuspended in 2 mL of sperm washing medium (human tubal fluid, HTF) and centrifuged for 7 min at 1600 rpm. The final sperm pellet was resuspended in 1 mL of HTF. Routine computer-assisted semen analysis and sperm function tests were done to examine the following parameters: total motility, percentage of sperm of hyperactivated motility, percentage of sperm with abnormal head shape, percentage of sperm with abnormal head size, motility (MOT), curvilinear velocity (VCL), linearity (LIN), amplitude of lateral head displacement (ALH), sperm motility after 60 minutes of separation, hypo-osmotic swelling (HOS) test, bovine cervical mucus penetration test, percentage viability by eosin-nigrosin staining test and sperm morphology differentiation by WHO and Kruger’s method. MOT, LIN, ALH, percentage recovery, HOS and viability were significantly better in sperm processed by Permash than MonoPercoll (P <0.05). Results of sperm morphology by WHO and Kruger’s criteria were similar between the two methods. In summary, discontinuous two-layer Percoll procedure provides significantly better semen characteristics than MonoPercoll sperm separation technique in normozoospermic men.

Materials and Methods: A total of 10 human ejaculates obtained for diagnostic semen analysis were each processed into three test groups. Group 1 was processed by gradient separation using Isolate™. Group 2 was processed using polyvinylpyrrolidone (PVP) coated beads (Percoll™, Pharmacia) and Group 3 using traditional swim up. The next ejaculate served as the control group. The resulting pellets were resuspended in Modified Ham’s F-10 with HSA and assessed according to WHO guidelines for sperm count, overall motility, forward progression, viability, acrosin activity and normal sperm morphology.

Conclusion: The results of this preliminary study show that Isolate™ is an equally effective method of sperm separation compared to currently used methods. Isolate™ as a new method for sperm separation: A microscopical sperm preparation technique may be a novel approach for intracytoplasmic sperm injection (ICSI). A. Hossain, S. Barik, B. Rizk, S. Low Jr, and L. Thomeyroth. University of South Alabama, Departments of ObGyn and Biochemistry, Mobile, AL.

Intracytoplasmic sperm injection procedure requires optimization of sperm and egg preparations before injection of a single sperm into egg. Notable simplifications have occurred in egg preparation, microsurgery, injection equipment and injection strategy. The sperm preparation, however, has not undergone any significant improvement. In ICSI procedure, the sperm are prepared by just about any method that yields a clean preparation. In this study we have developed a microscopical sperm preparation technique that is specially suited for ICSI. Sperm separation columns are used to separate sperm into three fractions: hyperactivated sperm, motile (MOT), non-motile (non-MOT) sperm. The separation is accomplished by using a 15 ml volume microgradient column that is constructed in the shape of a square with one open corner on a petri dish. One open end of the square contains semen, while the three other contain 15 ml variable concentrations of albumin used to maintain the gradient. The microgradient column, covered with mineral oil, was observed under an inverted microscope. When enough number of sperm reached the harvest zone, the column was harvested with a pipet tip. Twelve semen specimens with different male factor etiologies were processed with the column and the quality of the sperm yield was evaluated. Sperm preparation was successful in 12/12 (100%) semen samples. The motility of the prepared sperm in all samples was 100% although with grade A (WHO criteria). 96.6% sperm in the yield exhibited hypo-osmotic swelling. The percentage of spermatozoa with normal morphology (Kruger’s strict criteria) was 53.7%. The number of sperm reaching the harvest zone was correlated with the time in all samples. Five sperm injected into hamster oocytes exhibited swimming head (indication of decondensation). Microscopical sperm preparation does not induce any gross and ultrastructural physical damage to the sperm since the procedure does not require any pretreatment such as centrifugation of the semen. Furthermore, the technique is based on self migration of motile sperm in horizontal column, it always yields morphologically superior sperm with 100% motility from semen irrespective of its factor quality. The microscopical approach of sperm preparation therefore, might be an improved way of controlling the quality of sperm to be used in ICSI and thus improving the ICSI fertilization rate.


Sperm head size and shape are major determinants of normal sperm morphology which, when assessed using strict criteria, can reliably predict IVF success. We investigated whether these sperm characteristics are affected following preparation and subsequent incubation. Raw semen for IVF (n=72 specimens; 60 patients) was prepared using discontinuous Percoll columns followed by wash and swim-up. The prepared specimens were incubated for 24 and 48 hr at 37°C in 5% CO₂ in humidified air. Sperm motility was determined by CASA. Sperm morphology was evaluated using strict criteria; specific abnormalities were recorded for those sperm judged abnormal. Motility for raw, prepared, 24 and 48 hr specimens was 69.0±2.1%, 96.5±2.2%, 75.1±2.2% and 37.2±2.2%. The overall fertilization rate was 60.9±2.1%. Compared with the raw semen, the proportion of normal forms increased following preparation (22.0±5.6% vs. 17.3±0.6%, P<0.001), then decreased after 24 and 48 hr (15.9±0.6% and 15.3±0.6%, respectively). The incidence of abnormal midpieces, droplets and tails decreased after preparation, while the overall incidence of head abnormalities remained unchanged. The prevalent abnormal head shapes in raw semen were large oval (>3 μm long and/or >3 μm wide), asymmetric, pyriform, tapered, and amorphous. Although the incidence of most of these prevalent head abnormalities were unaffected by preparation, that of large ovals was increased (34.9±2.1% vs. 44.4±1.3%, P<0.001), but not further changed by incubation. In contrast, incubation resulted in an increased incidence of asymmetric heads (prepared:13.9±0.7% vs. 24 hr:19.1±0.6% and 48 hr: 19.2±0.6%, P<0.001 for both). Therefore, following preparation and during subsequent incubation, there was a reduction in the incidence of normal forms concomitant with an increase in the incidence of asymmetric heads. These changes appeared unrelated to sperm viability or fertilization. The present observations suggest that levels of large oval and asymmetric heads and the shift from normal forms to asymmetric heads may reflect normal, fertility-related events that occur in vitro, and possibly in vivo.
8.1 PROCESSING METHOD OF FROZEN DONOR SPERM CURRELPED WITH FINAL SPERM PARAMETERS AND INTRATRACHEAL INSEMINATION PREGNANCY OUTCOME

Of 4-49 intratracheal inseminations (ITIs) performed during a 12-month period, 102 used frozen donor sperm. The purpose of this study was to retrospectively compare the effectiveness of three protocols routinely used to prepare frozen/thawed sperm. To evaluate their significance on pregnancy outcome

4) patients with varying diagnoses of infertility underwent either natural cycle IVF or controlled ovulation by human menopausal gonadotropin (hMG). Once lead follicles reached >16 mm diameter, patients were given lCG (3,000 IU) and ITIs were performed approximately 35 h later with donor sperm previously matched and obtained from one of five commercial sperm banks. Semen was thawed at 24°C for 30 min and 37°C for 5 min. and the concentration of motile sperm and initial percent motility were measured by CASA. Initial preparations were performed by Swim-Up (SU). Percoll (P) or Sperm Wash (SW). SU samples were diluted 1:1 with Modified Sperm Wash Medium (Irvine Scientific. Irvine, CA) and centrifuged for 20 min at 100,000 g. The pellet was overlaid with 0.1 mL fresh medium and allowed to swim-up for 60 min at 37°C. The supernatant was collected, centrifuged and sperm were resuspended. P samples (1 mL aliquots) were placed undiluted on a discontinuous Percoll gradient of 95% and 75% centrifuged for 20 min at 500 x g and pelleted (0.5 mL). SW involved centrifugation of the whole sample for 15 min at 250 x g. removal of the supernatant and resuspension of the pellet as the final sample. Total sperm motility and percent motility were recorded by CASA. Patients returned in 2 wk for a blood pregnancy test. Statistical significance was determined using the Student's t-test.

There was no significant difference in initial sperm concentrations (motile sperm per mL) or initial percent motility prior to SU (P=0.01), P (n=21) or SW (P=0.01). However, the percent motility of the final sample was significantly different (p<0.001) between SU and SW, where SU samples had the highest motility (73.3% ± 20.3), which was not different from P samples (73.3% ± 22.3) and SW samples had the lowest motility (33.3% ± 17.7). There was a significant decrease in sperm motility when semen was frozen twice; however, when correlated to pregnancy outcome, SU preparation of sperm was the least superior, and P preparation was the most superior to SU preparation, as it was, despite the decrease in motility of the final sample. Therefore, based on the results of this study, swim-up and sperm wash procedures are recommended for preparation of frozen/thawed sperm.

8.2 FROZEN HUMAN SPERM MAINTAINS MOTILITY FOLLOWING PROCESSING AND REFREEZING

In general, human semen survives cryopreservation with enough efficiency to be used for IVF cycles. In some cases it may be necessary to reprocess the frozen sample for use in future cycles. In an effort to evaluate the survivability of sperm during this second freeze, samples were removed at successively steps of the washing procedure, evaluated for motility and progression (WHO standards) and re,frozen in TEST Yolk Buffer (Irvine Scientific) using a controlled rate freezer.

Frozen semen from several donors (from a local cryobank) was thawed (x), washed (y) with glass wool (z) and allowed to recover (a) from each step (minimum of 48 hour storage), then thawed for evaluation (x' y' z'). Results are reported in average change of motility percentage points during processing or refreezing, the mean percent motility ± standard deviation (sample pregnancy grade / average total motility) X and available (x', y', z'). In fresh specimens there were an average of 363 x 10^6 total sperm cells with a mean motility of 64.3 ± 2.5% (233). The average loss of motility in the initial freeze (x) was approximately 15 ± 1.5% (±5 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0, 100.0). Refreezing of the sample (x) after initial freezing caused the mean motility loss of 15.0 ± 2.5% (232). Retrieval of total cells during the washing procedure (y) was 89% (293 x 10^6). Yet washing the initial frozen sperm decreased the motility by 25 (22.8) percentage points, while the freeze-thaw protocol only increased the motility by 15.0 ± 2.5% (232). Retrieval of total cells following glass wool (z) was 17% (49 x 10^6). Processing the washed specimen through glass wool increased the sample motility by 35 (35.7) percentage points to 62.9 ± 2.5% (231), and refreezing this washed specimen (x') decreased the motility by an additional 5.0 ± 2.5% (230). Retrieval of total cells following glass wool (z') was 17% (49 x 10^6). In conclusion, processing frozen sperm may be refrozen. There are quantitative losses of total sperm cells during each processing step, and additional losses of motility occur following refreezing of these specimens. Yet, enriching the motility of the sample by processing a frozen specimen through glass wool prior to refreezing results in a survivability of this motile fraction similar to that of the initial frozen specimen.
Flow cytometric sperm sorting based on DNA is now a standardized method for sexing sperm. DNA is 3 to 4% greater in the X chromosome than the Y chromosome. Gender preselection in male animals can be predicted by virtue of the fact that DNA method can be validated by measuring the DNA of the sperm after separation. Since DNA is the only known difference between X and Y sperm, it constitutes the marker for the separation.

Hoechst 33342 a DNA binding stain is added to intact viable sperm to differentiate X from Y sperm. Prior to passing the sperm past the laser of the flow cytometer/cell sorter, sperm are incubated at 32°C for 40 minutes to attain uniform stain penetration. Procedures are effectively validated using reanalysis of the separated sperm for DNA content and sex at birth. Sexed sperm are then collected and recombined, rinsed with medium, and allowed to fertilize oocytes. Since DNA is the only known difference between X and Y sperm, it constitutes the marker for the separation.

Intra cytoplasmic sperm injection has also been reported by others to produce offspring of the predicted sex. Skewing of sex ratio has been reported in cattle, pigs, sheep, and rabbits. Purities recorded for sexed sperm have been reported to produce offspring of the predicted sex. Skewing has also been reported to produce offspring of the predicted sex.

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90 TESICULAR MICROLITHIASIS (TML) IN MEN WITH AZOOSPERMIA AND SEVERE OLIGOSPERMIA.

Departments of Urology, Radiology, Pathology, Jefferson Medical College, Philadelphia, PA.

Testicular malignancy has been associated with both the clinical spectrum of reproductive dysfunction and the ultrasound finding of testicular microlithiasis (TML). Yet few studies have investigated the association of TML in the infertile male population. This study’s goal is to establish the incidence of TML in the severely infertile male population.

A group of 18 men presenting with azoospermia or severe oligospermia (<5 x 10^6/ml) was scanned by high resolution scrotal ultrasound and 8 underwent testicular biopsy with PLAP stain for carcinoma in situ. Sonographic characterization of microliths included their number, size, laterality, and pattern of distribution. Based on clinical and laboratory parameters patients were characterized with a diagnosis of either genital tract obstruction or spermatogenic failure. Ultrasound detection of TML was present in 2 patients (spermaticogenic failure and genital tract obstruction). In the former, ultrasound characterization of TML was multifocal with 6 microliths ranging in size from 2-3 mm. Histologic correlation showed early spermatogenic arrest with tubular wall thickening and myoepithelial proliferation. In the latter patient a single ecchogenic focus of TML measuring 1-2 mm was noted. All of the specimens were negative for carcinoma in situ by PLAP stain.

The association of TML and azoospermia/oligospermia remains unclear at this early phase of study. Yet because of the detection of TML in this small initial cohort, periodic clinical and ultrasound monitoring is recommended in subfertile men with TML.

91 ARE SPERM MOTION PARAMETERS INFLUENCED BY VARICOCELE LIGATION?

Irvin H. Hirsch, Mohamed T. Ismail. John Sedor, Department of Urology, Jefferson Medical College, Philadelphia, PA.

Although there is no pathognomonic semen pattern induced by varicoceles, sperm motility has been shown to correlate most commonly with this clinical entity. In addition, all semen parameters, sperm motility is reported to improve most commonly following varicocele ligation. To objectively evaluate this hypothesis, computer-aided semen analysis (CASA) was utilized to assess sperm motion parameters in subfertile men before and after varicocele ligation. This study includes 34 men with varicocele as the primary cause of infertility who underwent physical examination, hormonal profile, and Doppler ultrasound. CASA was performed before and after either subinguinal or laparoscopic varicocele ligation. Patients were followed between 3 and 18 months postoperatively, and average CASA values were obtained pre- and postoperatively. Using the paired t-test, CASA values were analyzed with special reference to motility and sperm motion parameters.

Postoperatively, patients demonstrated increases in mean sperm density and in the overall distribution of sperm with rapid velocity, although not to statistically significant proportions. Increased mean values of straightness, linearity, and track speed were observed postoperatively, but only progressive sperm velocity increased to statistically significant levels (P < 0.008) postoperatively. Since progressive velocity has been a primary CASA parameter predictive of female fertility (Mathur, 1986) and successful assisted reproductive techniques (Holt, 1985), we conclude that varicocele ligation results in improvement in most semen parameters and, significantly, in the key parameter of progressive sperm velocity.

92 EFFECTS OF MULLERIAN INHIBITING SUBSTANCE ON HUMAN SPERM SURVIVAL.

Yong Sung, Mary E. Falletta, Fadia A. Attia*, Shelly C. Yaffe and Arnold M. Belker. Department of Surgery, University of Louisville and Louisville Andrology Laboratory, Jewish Hospital, Louisville, Kentucky.

Purpose: The use of cryopreserved sperm as a treatment for infertility is a major medical application of sperm banking. Upon thawing, however, sperm survival (viability and motility) is poor. This project investigates the effects of Mullerian inhibiting substance (MIS) on sperm survival in fresh specimens and after cryopreservation.

Methods: Spem motility in fresh semen (n=6) was assessed using Cell-Yu microscope counting slides after 0.5, 1, 3, 5, and 22 h incubation with and without (Control) MIS (0.5 μg/ml). Viability was assessed by eosin-nigrosin exclusion test. The same specimens cryopreserved in TES-tris-glycerol-egg yolk buffer (+MIS) were assessed by Cell-Yu microscope counting slides after 0.5, 1, 3, 5, and 22 h incubation with and without (Control) MIS for 2 weeks. The specimens were then thawed at 37°C, and survival assessed at similar time points. The specific effect of MIS was examined by co-incubation with anti-MIS monoclonal antibody (6E11) in fresh specimens (n=6). Statistical analysis was by Sign test, and a p-value of < 0.05 was considered significant.

Results:

<table>
<thead>
<tr>
<th></th>
<th>FRESH1</th>
<th>CRYOPRESERVED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>5 h</td>
</tr>
<tr>
<td>Motility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-MIS</td>
<td>71 ± 4</td>
<td>41 ± 9</td>
</tr>
<tr>
<td>+ MIS</td>
<td>71 ± 4</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Viability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-MIS</td>
<td>69 ± 5</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>+ MIS</td>
<td>70 ± 4</td>
<td>54 ± 6</td>
</tr>
</tbody>
</table>

1Sperm concentration (range): 22-76 x 10^6/ml; motility: 62-78%; normal morphology (WHO criteria) 15-31%; p < 0.03 compared with Control (+MIS). Co-incubation with 6E11 eliminated these effects of MIS. Conclusions: MIS improves sperm motility and viability, and thus may have potential for use in assisted reproductive technology.
The objective of this study was to compare the efficacy of intrauterine insemination (IUI) for male and/or cervical factor by age of female partner. A retrospective review of all patients who underwent IUI at a private practice of a university-based infertility center during an eleven month period in 1995 was undertaken. 281 patients who underwent IUI therapy for cervical and/or male factor were classified into two groups by age at time of their first IUI: younger than 40 years (n = 232), 40 years old or older (n = 49). Cervical factor was diagnosed based on a post-coital test that failed to show sperm with good forward progression as time of mature follicle. Male factor was diagnosed if the semen analysis demonstrated either low count, low motility, antisperm antibodies or subnormal hypo-osmotic swelling test. About half of the patients underwent IUI in natural cycles. The remaining patients underwent IUI following ovarian stimulation with either clomiphene citrate or low dose gonadotropins for the treatment of anovulation or follicular maturation defects. The main outcome measure compared was the cumulative probability of ongoing pregnancy (exceed 50%) achieved in the following 3 cycles of IUI was 28.6% for the younger group and 0.0% for the older group (p < 0.05). The age groups did not differ in terms of infertility history, indication for IUI, use of ovarian stimulation, number of follicles released and baseline semen parameters. The treatment of male and/cervical factor by IUI is ineffective for women 40 years old or older. This study reaches the same conclusion as did a previous reported investigation of IUI following superovulation. The study presented herein did not use superovulation techniques. Furthermore, all patients in the study presented herein had male or cervical factor whereas the majority of cases in the aforementioned study had unexplained infertility in the majority of cases.
Lubricants have been used to overcome vaginal dryness, to assist in the collection of semen and as a transcervical gel for ultrasound. We examined the toxicity of several lubricants (including one ultrasound gel) on Percoll-isolated sperm. Sperm (200 µl with a minimum of 50 million sperm/ml) were placed into tubes previously coated with approx. 20 mg of lubricant (KY-Jelly, Astroglide, Vaseline, Clear Image, and a control w/o no lubricant). Ten fresh semen specimens, in duplicate, were allocated to each treatment and a computer-aided semen analysis (Hamilton Thorne) was performed after 1 and 24 h exposure to the toxics. Clear Image, an ultrasound gel, was extremely toxic to sperm and in all but one specimen there were no motile sperm after 1 h exposure. Lubricants in order of toxicity were Clear Image, KY-Jelly, Astroglide and Vaseline. Interestingly, sperm exposed to Vaseline had significantly higher parameters (percent progressive motility, straight line velocity, lateral head displacement and straightness) than controls, sperm not exposed to lubricants (paired t-test: p < 0.05). KY-Jelly and Astroglide were toxic in the assay, but the clinical significance of this need to be examined. Clear Image is toxic and should not be used as a transducing gel prior to in vitro fertilization.

### Mean Values After 24 h

<table>
<thead>
<tr>
<th>Lubricant</th>
<th>% Motile</th>
<th>% Progressive</th>
<th>VSL (µ/sec)</th>
<th>ALH (µ)</th>
<th>STR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64</td>
<td>27</td>
<td>15</td>
<td>3.7</td>
<td>68</td>
</tr>
<tr>
<td>KY-Jelly</td>
<td>22*</td>
<td>9*</td>
<td>15*</td>
<td>1.5*</td>
<td>44*</td>
</tr>
<tr>
<td>Astroglide</td>
<td>37</td>
<td>14*</td>
<td>26</td>
<td>3.4</td>
<td>68</td>
</tr>
<tr>
<td>Vaseline</td>
<td>6*</td>
<td>1*</td>
<td>32*</td>
<td>4*</td>
<td>71*</td>
</tr>
<tr>
<td>Clear Image</td>
<td>2*</td>
<td>1*</td>
<td>9*</td>
<td>0.2*</td>
<td>*</td>
</tr>
</tbody>
</table>


different from Control: *p < 0.01; *p < 0.05; *p < 0.005.

There is a general opinion that the percentage of progressively motile sperm (PMOT) may be a more appropriate measure than the percentage of motile sperm in an assessment of sperm motion, but there is no standard definition of PMOT. With CASA, PMOT is generally defined as sperm exceeding a user-defined threshold path velocity (VAP) and/or straightness (STR). To examine the influence of different VAP and STR thresholds on PMOT, cauda epididymal sperm were collected from an untreated rat and allowed to disperse in Medium-199 at 36 °C. One aliquot was diluted and digital images collected on an optical disk using an HTM-IVOS (Hamilton Thorne Research, Beverly, MA) with the sample chamber at 36 °C. A second aliquot was “treated” by cooling to room temperature, diluted and recorded at 27 °C. Two hundred sperm images from each sample were analyzed at 60 Hz for 0.5 sec. The distributions of VAP and STR in each sample were compared, and cut-off values for defining PMOT were chosen. The control sperm were judged to be 95% progressively motile by manual assessment. The median VAP and STR of these sperm were 138 µ/s and 68, respectively. The median VAP and STR of the treated sperm were 119 µ/s and 36. Using a VAP threshold of 50 µ/s and a STR threshold of 30, gated out twitching sperm so that 96.0% of the control sperm and 59.5% of the treated sperm were defined as progressively motile. Raising the STR threshold to 50 yielded lower PMOT values (85.0% and 18.5% for the control and treated samples) for manual assessment, but provided a greater distinction between the treated and control sperm. Choosing the median VAP and STR of the control sperm to define PMOT selects for the fastest, straightest sperm. Using these cut-offs, 26.5% of the control and 1% of the treated sperm were defined as progressive. Thus, different definitions of PMOT may be used in CASA to differentiate between control and treated sperm populations, depending on the area of the distribution curves of VAP and STR that are of research interest.

### VASECTOMY-RELATED INFERTILITY: A COMMON AND EXPENSIVE MEDICAL PROBLEM

Anne M. Ignatius, King Edward Memorial Hospital, Perth, Western Australia 6008, Australia.

In the year 1995 in the State of Western Australia, some 1243 men underwent vasectomy while another 117 men (9.4%) underwent vasectomy reversal. Between January 1992 and December 1993, a total of 860 couples were seen by the author in an infertility clinic. Of these couples, 80 (9.3%) were found to have infertility that was related to a past vasectomy. Regret concerning vasectomy is thus a common problem. Of these 80 men, 73 were requesting treatment due to remarriage while the remaining 7 vasectomised men were in the same relationship but now wanted more children. The median age of the 80 vasectomised men was 42.7 years (range = 26-58 years) while that of their present partners was 32.4 years (range = 22-47 years). The vasectomy interval in this group varied from 1-26 years with a median vasectomy interval of 9.3 years. The pattern of seminal abnormalities was very diverse in these men but either azoospermia or oligo/asthenozoospermia were the most common abnormalities seen. Among the female partners of these vasectomised men, 13 (16.3%) were found to have pathology that would have caused infertility any semen problem in their partners. Among these 79, a total of 79 vasectomy reversals had been performed, while 73 cycles of donor insemination had been carried out, 3 of which were associated with ovulation induction. A further 18 cycles of IVF and IVF/ICSI had also been completed. The cost of this treatment has clearly been very high. A plea is thus made for far more careful counselling of men undergoing vasectomy and for the cryopreservation of semen pre-operatively. The initial application of epididymal aspiration of sperm and IVF/ICSI to this problem may now be the more successful and cheaper treatment options.

### PROGRESSIVE MOTILITY IN COMPUTER-ASSISTED SPERM ANALYSIS (CASA).


There is a general opinion that the percentage of progressively motile sperm (PMOT) may be a more appropriate measure than the percentage of motile sperm in an assessment of sperm motion, but there is no standard definition of PMOT. With CASA, PMOT is generally defined as sperm exceeding a user-defined threshold path velocity (VAP) and/or straightness (STR). To examine the influence of different VAP and STR thresholds on PMOT, cauda epididymal sperm were collected from an untreated rat and allowed to disperse in Medium-199 at 36 °C. One aliquot was diluted and digital images collected on an optical disk using an HTM-IVOS (Hamilton Thorne Research, Beverly, MA) with the sample chamber at 36 °C. A second aliquot was "treated" by cooling to room temperature, diluted and recorded at 27 °C. Two hundred sperm images from each sample were analyzed at 60 Hz for 0.5 sec. The distributions of VAP and STR in each sample were compared, and cut-off values for defining PMOT were chosen. The control sperm were judged to be 95% progressively motile by manual assessment. The median VAP and STR of these sperm were 138 µ/s and 68, respectively. The median VAP and STR of the treated sperm were 119 µ/s and 36. Using a VAP threshold of 50 µ/s and a STR threshold of 30, gated out twitching sperm so that 96.0% of the control sperm and 59.5% of the treated sperm were defined as progressively motile. Raising the STR threshold to 50 yielded lower PMOT values (85.0% and 18.5% for the control and treated samples) for manual assessment, but provided a greater distinction between the treated and control sperm. Choosing the median VAP and STR of the control sperm to define PMOT selects for the fastest, straightest sperm. Using these cut-offs, 26.5% of the control and 1% of the treated sperm were defined as progressive. Thus, different definitions of PMOT may be used in CASA to differentiate between control and treated sperm populations, depending on the area of the distribution curves of VAP and STR that are of research interest.
101 Mitocondrial DNA, semen quality and clinical assessment

Anne M. Axvig1, R. Martin2, Denise Mehnes2, J. Goldblatt2 & J.M. Cummins3. King Edward Memorial Hospital1, Division of Genetic Services, Princess Margaret Hospital2 & Murdoch University, School of Veterinary Studies3, Perth, Western Australia.

Male infertility has been likened to accelerated testicular aging and our preliminary results on testicular biopsies from infertile men has indicated strong links with mitochondrial (mt) DNA deletions (1,2). These findings are consistent with models of aging and tissue degeneration based on progressive mtDNA dysfunction (3).

We have studied 66 semen samples from 64 men attending an infertility clinic and have used semi quantitative PCR to amplify control regions of the mtDNA genome as well as spanning the "common" 4977 basepair deletion. The deletion was classified as mild (<0.03%), moderate (0.03-0.09%) or severe (>0.09%).

Deleted mitochondrial DNA was detected in 34 of the 66 (52%) samples studied. Of the 34 patients with deletions in the cells of their seminal fluid, 8 were mild, 15 were moderate and the remainder were severe. In only 1 of these 34 men could pathology be detected clinically but of these men with evidence of deletions, 4 were oligozoospermic (WHO Classification) and 1 was azoospermic. Of the men where no significant deletions could be found in their semen, 3 were oligozoospermic and 2 were azoospermic due to germinal aplasia.

It is clear that the presence of a mtDNA deletion in semen bears little relationship to the aetiology of the infertility and thus seminal fluid cannot be used to detect such lesions. These investigations must thus be pursued in testicular tissue rather than semen.


Therefore, computer-assisted sperm motion analysis (CASA) in rodent reproductive toxicology studies has been conducted on freshly epididymal sperm functional capacity, we are exploring the feasibility of using CASA during in vitro capacitation to monitor the hyperactivated motility associated with fertilizing ability. Hamster epididymal sperm were capacitated in vitro and sampled at intervals for CASA analysis using the HTM-IVOS (Hamilton Thorne Research, Beverly, MA). Sperm were imaged in 100 µ depth flat cannules using a 4x objective and pseudo-dark field optics. Images were stored to optical disc and recalled for analysis at 60 Hz for 0.5 sec. (minimum contrast = 40, minimum size = 0). After verifying tracking accuracy during playback, sperm tracks were printed and categorized visually as "progressive" (Prog), "transitional" (Trans) or "hyperactivated" (Hyper) in accordance with previous descriptions of hamster sperm motion (Suarez et al., 1984). Mean CASA parameters of ten representative tracks for each category are listed below, along with the % acrosome reaction (AR) at the time of analysis.

<table>
<thead>
<tr>
<th>Path</th>
<th>VSL</th>
<th>VAP</th>
<th>VCL</th>
<th>STR</th>
<th>LIN</th>
<th>ALH</th>
<th>BCF</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prog (0.5h)</td>
<td>173</td>
<td>238</td>
<td>434</td>
<td>74</td>
<td>43</td>
<td>23</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Trans (1.25h)</td>
<td>207</td>
<td>272</td>
<td>673</td>
<td>76</td>
<td>31</td>
<td>33</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Trans (3h)</td>
<td>206</td>
<td>293</td>
<td>678</td>
<td>71</td>
<td>31</td>
<td>35</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Hyper(3h)</td>
<td>158</td>
<td>348</td>
<td>783</td>
<td>45</td>
<td>20</td>
<td>43</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Hyper (4h)</td>
<td>138</td>
<td>380</td>
<td>861</td>
<td>36</td>
<td>17</td>
<td>40</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

These data document the dramatic increases in sperm velocity and vigor that accompany capacitation and the decrease in linearity associated with hyperactivation. Such data can now be analysed with multivariate methods to develop integrated models for defining hyperactivated sperm tracks. Supported by NIH ES03614. This abstract does not necessarily reflect EPA policy.

103 A NEW CATEGORY OF INFERTILITY DUE TO SPERM-SPECIFIC ABNORMALITY OF CD46

M. Kitamura, K. Matsumiya*, M. Yamanaka*, A. Okuyama, Department of Urology, Osaka University Medical School, Osaka, JAPAN and T. Seya*, Department of Immunology, Center for Adult Diseases, Osaka, JAPAN

Three infertile subjects fulfilling normal or subnormal criteria on routine semen analysis showed abnormal sperm CD46 (membrane cofactor protein of complement) by SDS-PAGE/immunoblotting analysis using a panel of monoclonal antibodies. These patients were screened from 63 male infertile patients with normal semen parameters, using Acro beads test, which utilize agglutination reaction with anti-CD46 antibody. The sperm CD46 isoform has been reported to be associated with sperm-egg interaction. These 3 patients expressed normal CD46 isoforms on lymphocytes and granulocytes. Thus, sperm-specific abnormalities in these proteins parallels male infertility, suggesting a new category of infertility, probably due to aberrations in the molecules related to sperm-egg interaction.

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The objective of this study was to assess the efficacy of two delivery systems containing spermicidal formulations consisting of nonoxynol-9 (N-9) coprecipitated with polyvinylpyrrolidone (PVP) or PVP-N-9, and their ability to prevent the onset of pregnancy in rabbits. Semen specimens from 8 male rabbits (New Zealand White) were collected, pooled, assayed and utilized for the artificial inseminations (AI). Thirty-two doses were allocated into 4 Groups. Spermaticidal formulations were delivered in the form of Capsules or Tablets (vaginal spermicidal vehicles). Dose in Group 1 (control) received a placebo Tablet or Capsule, vaginally. Doses in Groups 2 to 4 received an experimental Tablet or Capsule, vaginally. Doses in Group 1 were inseminated at 0-h post-insertion of Capsules or Tablets containing no spermicides (PVP only). Doses in Groups 2, 3 and 4 were inseminated (vaginally) at 0.5, 0.5-h post-insertion of Capsules or Tablets containing spermicides (PVP-N-9), respectively. The insemination dosage consisted of 0.5 mL containing 70-80 x 10⁶ spermatozoa with 70-80% motility. The inseminated doses were induced to elevate 6-b (IM injection: 200 U HCG) prior to the time of insemination. The pregnancy rates (PR's) obtained from the mating study are shown below:

<table>
<thead>
<tr>
<th>Delivery system</th>
<th>0-time placebo</th>
<th>0-time experimental</th>
<th>0.5-h</th>
<th>6-h</th>
<th>experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule</td>
<td>75%</td>
<td>75%</td>
<td>25%</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>Tablet</td>
<td>75%</td>
<td>50%</td>
<td>0%</td>
<td>22%</td>
<td></td>
</tr>
</tbody>
</table>

The results showed that both delivery systems exhibited some variations in PR's over the various time intervals (experimental) compared to the control values (placebo at 0-time). Considering both delivery systems, the PR's were reduced the most at 0.5-h post-insertion of the delivery system. Most interestingly however, the Tablet seemed to be a more efficient delivery system than the Capsule, yielding significantly lower PR's (21% to 50%) than the Capsule throughout the 3 time intervals assessed. Also, when the Tablets and Capsules PR's were compared with the placebo results, the Tablet PR's yielded consistently lower PR's at all 3 time intervals where the Capsule PR's were lower only at 0.5-h post-insertion. The Tablet formulation-delivery system, as applied in this study, was found to be the most efficient mode of delivery of the tested spermicidal formulation (Supported by NICHD Contract No. NOI-HD-3-184).


We have previously shown that both delivery systems exhibited some variations in PR's over the various time intervals (experimental) as compared to the control values (placebo at 0-time). When considering both delivery systems, the PR's were reduced the most at 0.5-h post-insertion of the delivery system. Most interestingly however, the Tablet seemed to be a more efficient delivery system than the Capsule, yielding significantly lower PR's (21% to 50%) than the Capsule throughout the 3 time intervals assessed. Also, when the Tablets and Capsules PR's were compared with the placebo results, the Tablet PR's yielded consistently lower PR's at all 3 time intervals where the Capsule PR's were lower only at 0.5-h post-insertion. The Tablet formulation-delivery system, as applied in this study, was found to be the most efficient mode of delivery of the tested spermicidal formulation (Supported by NICHD Contract No. NOI-HD-3-184).

105 CYPROTERONE ACETATE (CPA) PLUS TESTOSTERONE ENANTHATE (TE) 100 mg/week INDUCED A DOSE-DEPENDENT SUPPRESSION OF SPERMATOGENESIS IN NORMAL MEN. MC Meriggiola, C. Flamigni, WJ Brenmer. University of Bologna, Bologna, Italy and University of Washington, Seattle, WA.

Previous studies of hormonal male contraception have resulted in inconsistent suppression of spermatogenesis and side effects such as decrease of HDL-cholesterol that would greatly hinder the acceptability of the contraceptive. Recently, we tested whether the combination of physiologic doses of TE with CPA could induce a more profound sperm suppression and avoid androgen related side effects. Twenty-five normal fertile men were divided into 5 groups of 5 men each and treated with TE 100 mg/week alone or in combination with CPA at the dose of 100 mg/day (CPA-100), 50mg/da (CPA-50), 25 mg/da (CPA-25), and 12.5 mg/da (CPA-12.5). The study consisted of a control phase, treatment phase lasting 16 weeks and a recovery phase lasting until each subject had at least one sperm count within his own baseline range. During the study phases, seminal fluid analyses were performed every 2 weeks and blood samples were drawn every 4 weeks for measurements of chemistry tests. RESULTS: Regardless of the dose, the combination of CPA plus TE was more effective in suppressing sperm production than TE alone: azoospermia oligospermia oligospermia

1. CPA

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109  TRIPOTOLIDE: A POST-TESTICULAR ANTIFERTILITY AGENT
Y.H. Lee*, A.P. Sinha Hikim, A. Leung*, S. Baravarian*, C. Wang, and R.S. Swerdloff, Dept. of Medicine, Harbor-UCLA Medical Center, Torrance, CA.

The antifertility effect of tripotolide (TW), isolated from Tripterygium wilfordii, has been demonstrated in male rats and mice, as well as in men. TW has been proposed to work at a post-testicular level but additional antispermatic effects remained possible. Our objective was to determine whether this compound at selected dose levels that induce infertility have anti-spermatic effects. Groups of six male adult SD rats were given oral administration of either vehicle (control); C) or tripotolide (50 or 100 µg/kg BW) daily for 35 or 70 days. Body weight gain was normal in all treated groups. All six rats treated with high dose of tripotolide were sterile during the second (days 63-70) mating trial (pregnancy rate with exposure to 2 females per male was TW: 0/11 vs C: 10/10). Lower dose (50 µg) of TW gave intermediate values (pregnancy: TW: 8/12 vs C: 10/10). Plasma levels of LH, FSH, and T were indistinguishable from those of the C. No effects of tripotolide were observed on tests and accessory organs or sperm numbers of tubular lumens and Leydig cells, tubule diameter, and homogenization-resistant advanced spermatic numbers. There was a modest decrease (p<0.05) in tubule volume. In situ 3'-end labeling of apoptotic DNA fragmentation, which permits recognition of dying cells at a very early stage of degeneration that could have escaped detection in routine histological procedure, further confirmed our morphological findings of no effects of tripotolide on spermatogenesis. In striking contrast, cauda epididymal sperm concentrations were decreased by 60.0% and the number of immotile sperm in semen was mixed with 0.2% of a solution of tetramethylrhodamine/HEO (TMB) in dimethylformamide (Diachex Inc., Boston, MA), incubated at room temperature (500 minutes) and the number of peroxidase-positive cells exhibiting a blue color scored by brightfield microscopy. Leukocyte count in semen was also determined using the LeukoScore™ kit as follows: 50µl of the liquefied semen was added to a 3 mm-diameter well in a plastic module and allowed to drain through a filter (2.7 µm in pore size) sandwiched in the plastic module, followed by addition of 50µl of the TMB reagent. The absence of color on the filter following addition of the TMB reagent was considered a negative test (< 1 million/ml) and the presence of blue color was considered a positive test (> 1 million/ml). Of the 128 samples evaluated, 32 had leukocyte counts > 1 million/ml (1 to 3 million/ml) and 96 had counts < 1 million/ml (0.02 to 0.85 million). All semen samples with leukocyte counts < 1 million/ml were correctly identified as negative by the LeukoScore™ kit. Of the 32 samples with counts > 1 million/ml, 31 were correctly identified as positive and 1 was incorrectly identified as negative. Leukocyte count in this sample was 0.90 million/ml. The sensitivity, specificity and positive and negative predictive values of the LeukoScore™ kit for leukocyte count at the 1 million/ml cutoff point were 100%, 99%, 97% and 100%, respectively. Total test kit time was 1 minute. These results indicate that the LeukoScore™ kit could be used as a convenient tool for the diagnosis of leukocytospermia in the andrology laboratory, in the physician's office and potentially at home.


Determination of sperm count and percent motility in semen by microscopic analysis is a commonly used test for the diagnosis of male factor infertility. In addition, the determination of sperm viability is often used as part of the routine semen analysis performed in the andrology laboratory. The objective of this study was to evaluate the performance of the novel VitalScore™ kit in the determination of sperm count and viability in semen, as compared to microscopic analysis. A total of 50 semen samples were obtained from 50 males presenting for infertility screening. Aliquots of the liquefied semen were scored for sperm count and percent motility, and percent viability by phase-contrast and brightfield microscopy. Sperm count and sperm viability in semen were also determined using the VitalScore™ kit as follows: Aliquots of 50µl of the liquefied semen were added to wells #1 and #2 in a 2-well plastic cartridge and allowed to drain through a filter sandwiched in the cartridge. Then, 50µl of a 0.1% solution of Triton X-100 in PBS and 50µl of a propidium iodide solution in PBS (1 mg/ml) was added to well #1 and 50µl of propidium iodide alone added to well #2. Both wells were rinsed with 50µl of distilled water. The color produced on the wells was then scanned with a densitometer and the integration areas used for quantification. The color produced in well #1 was used to obtain total sperm count and the color produced in well #2 was used to obtain the number of permeabilized sperm. The integration areas were compared to the results obtained by phase-contrast and brightfield microscopy using regression analysis. Sperm count in semen ranged between 10 and 80 million/ml, percent motility between 10 and 90 and percent viability between 12 and 95, as determined by microscopic analysis. A significant correlation was found between sperm count and propidium iodide-stained sperm pretreated with Triton X-100 (r = 0.96). The number of spermatozoa stained with propidium iodide in the absence of Triton X-100 was highly correlated with the number of permeabilized sperm (r = 0.98) and with the number of immotile sperm in semen (r = 0.96). Total test kit time was 2 minutes. These results indicate that the VitalScore™ kit could be used as a convenient and reliable test for the determination of sperm count, sperm viability and sperm motility in semen.
SPERM DENSITY BUT IS NOT AS SENSITIVE FOR LOW DENSITY

Home kits for determination of male fertility potential has recently been introduced commercially.

Objective: To evaluate the FertilitySCORE™ test with our regular semen analysis.

Methods: One hundred and eighteen consecutive samples received for routine semen analysis were included in the study. Sperm density was assessed microscopically using a hemocytometer and expressed as million/mL. FertilitySCORE™ test results ranged from 1-6 for negative tests and 7-14 for positive tests. For comparison purposes the cutoff for sperm density was taken as 20 million/mL and the corresponding cutoff for the FertilitySCORE™ test result as 6. Nine samples could not be tested with FertilitySCORE™ because of low volume.

Results: Fifty nine samples had a negative FertilitySCORE™ test, of which 13 were equal or lower than 20 million/mL. The remaining 50 samples had densities greater than 20 million/mL and all had positive FertilitySCORE™ test scores. The sensitivity of the test was 22%, specificity 100%, PPV 100% and NPV 52%.

Conclusions: A positive FertilitySCORE™ test is indicative of 'good' semen sample, i.e., normal sperm density. The kit cannot be used for low volume semen samples and a negative test should be repeated.

Sperm concentration and motility are not sufficient in the accurate diagnosis of the patient's fertility potential. Assays that probe the functional activity of the spermatozoa are important. Occurrence of acrosome reaction and the recognition of zona pellucida by the head directed mannose receptors on the spermatozoa are two important steps in fertilization. The aim of the study was to evaluate the correlation between mannose ligand receptor (MLR) assay and acrosome reaction (AR) as well as between the above tests and sperm motility and morphology:

Semen samples from 14 normal volunteers were analyzed by computer assisted semen analyzer and morphology smears by Kruger's method. Spermatozoa were assayed after 6 and 24 h of capacitation at 37°C in Ham's F-10 medium containing 3% human serum albumin (HSA). Hoechst-33258 (1µg/mL) was used to assess viability. Appearance of head directed surface mannose receptors were detected by the binding of fluorescein isothiocyanate labeled (FITC) mannose-labeled bovine serum albumin. Acrosomal status was simultaneously evaluated by rhodamine (RTIC)-labeled *pisum sativum* lectin. FITC and RTIC Type III patterns were similar at 6 and 24 h of capacitation. A strong agreement was seen between these patterns at 6 h (r = 0.80, P < 0.0007) and at 24 h (r = 0.80, P < 0.0008). Sperm morphology showed no significant correlation with MLR and AR binding patterns at both time periods. However, a good correlation was seen between motility and MLR (r = 0.65, P <0.01) at 6 h and between motility and AR (r = 0.66, P < 0.01) both at 6 and 24 h (0.51, P <0.06). Sperm morphology can not adequately predict the functional status of the spermatozoa. MLR assay is a reliable and inexpensive test, it is easy to interpret and may be used effectively in the assessment of male fertility potential.

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LIPID PEROXIDATION IN CRYOPRESERVED SEMEN FROM CANCER PATIENTS. Y. Wang, R.K. Sharma, A.J. Thomas, and A. Agarwal. Andrology Research and Clinical Laboratories, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH 44195

Preserving the fertility by cryopreserving semen specimen before chemotherapy, radiation therapy or surgery is a realistic option for cancer patients. Higher level of lipid peroxidation can adversely affect semen quality in men with male factor infertility. Although the prefreeze and postthaw semen quality in cancer patients is poor, it is not clear if lipid peroxidation (LPO) affects the semen quality. Therefore, the present study was designed to compare lipid peroxidation levels in prefreeze and post-thaw semen specimens at concentrations of less than 20x10^6/m. In pre-freeze specimens, higher sperm counts were seen compared to HTM (P <0.02) as compared to the manual results. However, lower sperm counts were seen in the post-thaw specimens (P <0.01). The percentage difference between CASA and manual results was significant in cancer patients (P = 0.011) compared to normal donors, and these differences were higher in cancer patients (HTM and MA, P <0.0001). In post-thaw specimens, MA motility results showed no significant percentage difference with manual motility, whereas, significant differences were seen between manual motility and HTM motility (P <0.0008). HTM analyzer in general gave lower motility than the manual method while MA gave higher readings. CASA results are unreliable at sperm counts of >20x10^6/mL and when motility is less than or equal to 10%. They show minimum percentage difference from the manual results at sperm counts of greater than 80x10^6/mL. Manual verification of CASA results is necessary due to the lack of accuracy of CASA readings.
121 DIFFERENT TECHNIQUES TO IDENTIFY AND LABEL HUMAN ROUND SPERMATIDS

Introduction: When fully developed spermatozoa are absent in patients with non-obstructive azoospermia, the injection of a spermid by ICSI is the only therapeutic option. The aim of this study was to develop means of identifying and labelling haploid cells in testicular biopsies from azoospermic men. Their ploidy was confirmed by cytogenetic analysis of individual cells.

Material and Methods: Testis biopsies of -500 mg were divided into small pieces and tubules were disrupted by homogenization, or manually with fine forceps or needles. To assess the tissue for the presence of spermatozoa, 5 µl aliquots of the resulting suspensions were smeared on pre-stained slides and studied by bright field microscopy at 1000x, on phase contrast microscopy at 400-600x. and also by acrosin immunolabeling using FITC-anti boar acrosin antibodies. In order to isolate individual round spermatids, cells were selected from 3 µl of the suspension placed under oil with a glass micropipette of 7 µm inner diameter controlled by a micromanipulator at 400x. The cells were then analyzed on a microscope by fluorescence in situ hybridization (FISH) for chromosomes 18, X and Y.

Results: A total of 16 biopsies with different degrees of spermatogenic arrest were processed by dissection with fine forceps or needles. In addition, tissues from three patients were homogenized. The most reliable method for disruption of tubules while maintaining the integrity of the putative spermids proved to be manual dissection. Round spermatids were identified as a cells of 6-7 µm in diameter, with an acrosomal granule or cap and displaying an intact plasma membrane. Spermatsids were isolated from 12 biopsies using light microscopy and the acrosin assay as a marker, and these were detected in 3 of 5 patients with a complete absence of spermatozoa. The incidence of round spermatids in the different biopsies ranged from 1-17% of the total cells scanned. In order to confirm their ploidy, individual cells were isolated from four biopsies by micromanipulation, with a total of 7 slides being processed for FISH analysis. Due to a high rate of loss, only 19 cells were assessed but all displayed a signal, and 17 were haploid.

Conclusions: Spermatozoa or round spermatids were found in ~70% of men with non-obstructive azoospermia. In the complete absence of spermatozoa, round spermatids were isolated from 3 of 5 cases. Thus, cell diameter and the presence of the precursor for acrosin appear to be useful markers through which to identify round spermatids.

122 CRYOPRESERVATION OF SPERM COLLECTED BY TESE PROVIDES ADEQUATE POST-THAW VIABILITY AND SUCCESSFUL IVF-ICSI PREGNANCIES. R. Dolgina, G Wolf*, P. Studney*, B. Kaplan*, C. Niederberger, L. Ross, G S Prins. Department Urology, University of Illinois, College of Medicine, Chicago, IL; Reproductive Genetics Institute, Illinois Masonic Medical Center, Chicago, IL

Testicular sperm extraction (TESE) combined with IVF-ICSI is a new therapeutic modality for patients presenting with azoospermia due to various etiologies. TESE and IVF-ICSI procedures are commonly scheduled and performed concurrently to allow for the use of fresh sperm during ICSI. However, simultaneous scheduling presents several drawbacks particularly when there is inadequate sperm or egg extraction to allow for CPI, or cap and displaying an intact plasma membrane. Spermatids were isolated from 12 patients who underwent TESE followed by sperm cryopreservation. The mean recovered sperm count per patient was 2.13 ± 0.53 million following unilateral or bilateral testicular extraction. While motility was low or absent, eosin stain revealed 62 ± 4% viable cells. Sperm were subsequently frozen in TEST-yolk buffer and stored at -196°C. A sample aliquot was assessed for post-thaw viability which averaged 27 ± 2%. Only two patients had zero viable cells post-thaw and of those, one achieved pregnancy following ICSI. Thus we conclude that in most situations, enough viable sperm are available post-thaw for use in ICSI. Seven patients underwent IVF-ICSI using thawed TESE sperm and all cycles resulted in successful fertilization. Seventy-one oocytes were injected; 46 showed normal fertilization (65%) and 33 exhibited normal cleavage divisions (72%). Four viable pregnancies ensued (three singletons, one twin) with one normal delivery and three ongoing. This 57% pregnancy rate using frozen-thawed testicular sperm documents the efficacy of cryopreservation for sperm collected at TESE.


To determine the potential of a genome resource bank for cheetah conservation, we assessed the feasibility of collecting sperm from wild individuals then transporting and infusing this material into the genetically stagnant captive population. Our objectives were to: 1) collect cryopreserved sperm from wild-caught cheetahs in Namibia; 2) assess cheetah sperm survival post-thaw; 3) determine the efficacy of using laparoscopic intrauterine artificial insemination (Al) with cryopreserved cheetah sperm for infusing wild genes into the North American cheetah breeding program. Electroejaculates from 11 Namibian cheetahs were assessed for % sperm motility (M), % normal sperm (NS) and % non-intact acrosomes (NA). Semen was washed in Ham's F10 + 5% fetal calf serum and pellets resuspended in PVD cryodiluent (20% egg yolk, 11% lactose, 4% glycerol). Samples were cooled (30 min, 4°C), pelleted on dry ice and stored in liquid nitrogen for shipping. After thawing (37°C), aliquots were assessed for M and NA. Ten AI procedures were conducted in 9 females treated with 200 IU eCG/100 IU hCG. Mean pre-freeze M, NS and NA were 69.4%, 20.7% and 12.8%, respectively, which were similar to semen traits observed in other populations of free-ranging and captive cheetahs. The PVD/pellet method provided cryoprotection for sperm motility (mean post-thaw M, 47.5%); however, a high incidence of acrosomal damage was detected after thawing (mean post-thaw NA, 59.5%, range, 44-68%). Two females inseminated in utero with frozen-thawed sperm (6 and 15 x105 motile sperm/Al) became pregnant and produced 1 and 3 cubs, respectively. These results demonstrate the: 1) feasibility of collecting and cryopreserving sperm from 'wild' cheetahs; 2) susceptibility of cheetah sperm to acrosomal damage during freezing; 3) biological competence of frozen-thawed cheetah sperm; and 4) potential of a genome resource bank and Al for gene infusion in captive cheetahs. (Funded by British Airways and a grant from 12 zoological institutions in North America)
The combination of fluorescent staining and flow cytometry provides for a rapid and precise assessment of the functional status of thousands of individual sperm. We sought to quantify two seminal characteristics, mitochondrial function and sperm viability, using fluorometric techniques and compare these with the classical microscopic measurement, sperm motility. Fluorescent staining of sperm with active mitochondria was accomplished using rhodamine 123 (R123), 5,5',6,6'-tetrachloro-1',3',3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) and MitoTracker Green FM (MIT). These metabolic measurements were compared to those found with microscopic assessments of sperm motility (progressive sperm motility) initially (MOT0) and after 3 hr at 37°C (MOT3) and with a fluorescent assessment of sperm viability (SYBR-14 and propidium iodide [PI] staining). Cryopreserved sperm samples from each of 12 bulls were thawed, fluorescently stained and quantified by flow cytometry. Significant differences (P<0.01) were found among bulls for each of the mitochondrial function measures, but not among the metabolic measurements. The mean percentages (n=3) of progressively motile sperm initially (MOT0) ranged from 27 to 83 for the 12 bulls. After 3 hr this range decreased to 2 to 33% (MOT3). From 4 to 70% of the sperm were viable (stained with SYBR-14). The proportions of sperm staining with R123 ranged from 2 to 67% while MIT fluorescence was exhibited in 4 to 71% of the sperm. The percentage of sperm staining with JC-1 was also similar ranging from 4 to 68. The three fluorescent measures of mitochondrial function, R123, JC-1 and MIT, were correlated with MOT0, r = 0.96, 0.87 and 0.96 and MOT3, r = 0.96, 0.86 and 0.96, respectively. The mitochondrial measurements also were correlated with SYBR-14 staining, r = 0.98, 0.93, 0.96 for R123, JC-1 and MIT, respectively. These data indicate that the metabolic activity of individual cryopreserved bovine sperm can be readily quantified using a variety of mitochondrial-specific fluorescent stains. (Supported by USDA grant 95-37203-2186.)
We invite you to join the American Society of Andrology!

Introduction

Founded in 1975, the American Society of Andrology (ASA) is composed of clinicians and basic research scientists involved with the physiology and pathology of male reproductive functions. The research interests of andrologists encompass several basic science and clinical disciplines including biochemistry, urology, immunology, psychology, genetics, gynecology, pathology, pharmacology, internal medicine and animal science. The Society fosters a multidisciplinary approach to the study of male reproduction and sexual function.

Statement of Purpose

The Society functions to promote scientific interchange and knowledge of the male reproductive system and to advance the understanding of andrology through annual meetings, postgraduate courses and the publication of meritorious studies in the Journal of Andrology.

Journal of Andrology

Published bimonthly, ASA members receive the Journal of Andrology, a major source of scientific reports and topical reviews covering all aspects of male reproduction and sexual function. The Journal continues to be the strongest journal in the field of andrology based upon impact factor and its contents are indexed in Current Contents and Index Medicus. A $175 value, the journal is provided as a part of membership.

Annual Meeting and Postgraduate Course

ASA’s Annual Meeting offers an unequaled opportunity to learn about the latest advances in basic and clinical andrological research in the form of symposia, lectures and research reports (oral and poster). The meeting is designed to serve the interests of ASA’s diverse membership and state-of-the-art lectures supported by industry attract prominent speakers to accomplish this task. The annual Andrology Laboratory Workshop, Women In Andrology Luncheon, Student Colloquium and Awards Presentation are also featured events. Continuing medical education credits (CMEs) are offered to qualified attendees of the annual meeting and postgraduate course. Members receive early notice and lower registration fees for the Annual Meeting and Postgraduate Course. Upcoming meeting locations include Baltimore ('97), Long Beach ('98) and Chicago ('99).

ASA On-Line: Androlog

ASA members have access to Androlog...the online forum for discussing the latest issues and topics in andrology with colleagues from around the world. The Society also maintains a Home Page which can be used to find out the latest association news as well as an e-mail link to our administrative offices to request a wide variety of information.

ASA Newsletter

The ASA Newsletter is circulated 2-4 times per year and updates members on the many activities of the Society as well as news on NIH and private funding.

Student Benefits

Students are the future of any research field — and Andrology is no different. With this in mind, the ASA offers special student discounts on membership and registration fees for the high quality postgraduate training programs that are offered by the society. A student newsletter is mailed to all student members in conjunction the ASA newsletter. Travel funds are available for qualified students who present research at the Annual Meeting.

Other Membership Benefits

Membership Directory...Includes all ASA members, listed and referenced by name, location, research interests and specialities.

Handbook of Andrology...An 81-page soft-bound handbook that presents the basic science and clinical aspects of the male reproductive system. Free for ASA members.
### Membership Application

**INSTITUTION Mailing Address**

**Title**

**Department**

**City, State, Zip Code, Country**

**Phone**

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**Email**

**EDUCATION**

*Undergraduate, graduate, postdoctoral - in chronological order*

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<tr>
<th>Institution</th>
<th>Degree</th>
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**SPECIALTY** (circle one)

- **BR** Basic Research
- **U** Urology
- **E** Endocrinology
- **OG** Obstetrics/Gynecology
- **IM** Internal Medicine
- **P** Pediatrics

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- **a** Advanced Laboratory Diagnostic Tech.
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- **f** Electroejaculation
- **g** Cellular Biology/Sperm Physiology
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- **i** Infectious Diseases
- **j** Infertility
- **k** Oncology
- **l** Nutrition
- **m** Sperm Anatomy/Biology
- **n** Testis Anatomy/Biology
- **o** Accessory Glands
- **p** Toxicology
- **q** Endocrinology
- **r** Contraception
- **s** Microsurgery
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- **Student**
- **Emeritus**

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- Completed form
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  - Residents of North America: $100/yr $30/yr $2,000
  - residents outside of North America: $110/yr $40/yr $2,200

- Surcharge to receive Journal of Andrology by Air Mail:
  - Mexico or Canada: $12
  - South America, Europe or North Africa: $28
  - South Africa, Middle East, Australia, New Zealand or Asia: $35

- Letter from advisor for any student member, stating that he/she is a student seeking any degree or is a postdoctoral fellow, intern or resident.

- Signature of one current ASA member or one letter of recommendation.

**Signature of Sponsor**

**Signature of Applicant**

**Date**

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- **ACTIVE MEMBER**
  - Open to any qualified physician or scientist with an interest in the field of andrology.

- **LIFE MEMBER**
  - Open to any active member who makes a single payment equivalent to twenty (20) years of dues to the Society.

- **STUDENT MEMBER**
  - Open to any student in a program leading to a degree, a post-doctoral fellow, a medical intern or resident interested in the progress and development of andrology.

- **EMERITUS MEMBER**
  - Open to any member who is retired and is worthy of the designation of Emeritus.