MARCH/APRIL 2007
Supplement

Journal of ANDROLOGY

American Society of Andrology

32nd Annual Meeting

April 18–24, 2007
Tampa, Florida

Program and Abstracts

Published by THE AMERICAN SOCIETY OF ANDROLOGY

schedule at a glance

XIX North American Testis Workshop
"Chromosome Structure and Gene Expression"
April 18 - 21, 2007

Andrology Lab Workshop
"Sperm Morphology -A Hands-On Workshop"
"Quality Control -A Hands-On Workshop"
April 21, 2007

ASA Special Symposium
"Androgen Deficiency in Men - Challenges and Solutions"
Co-Chairs: Ridwan Shabsigh, MD and Ronald Swerdloff, MD
April 21, 2007

ASA 32ND ANNUAL MEETING

On The Cutting Edge of Basic, Translational and Clinical Andrology: Implications for the Future of Male Reproductive Health
Program Chair: Alvin M. Matsumoto, MD
April 21 – 24, 2007
**Location: Regency Ballroom 4-7 (unless otherwise noted)*

Registration fee includes entry into the lectures, one ticket to the Welcome Reception, a syllabus, and refreshment breaks.

FRIDAY, APRIL 20, 2007

7:00 a.m. – 5:00 p.m. **Registration**
Location: Registration Counter, Ballroom Level

4:00 p.m. – 11:00 p.m. **Executive Council Meeting**
Location: Buccaneer A-B

SATURDAY, APRIL 21, 2007

7:00 a.m. – 7:30 p.m. **Registration**
Location: Registration Counter, Ballroom Level

4:00 p.m. – 7:00 p.m. **Exhibit Hall Open**
Location: Regency Ballroom 1-3

6:00 p.m. – 6:10 p.m. **Welcome and Opening Remarks**
President: Christina Wang, MD
Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute

Local Arrangements Chair:
Don F. Cameron, PhD
University of South Florida

Program Chair:
Alvin M. Matsumoto, MD
University of Washington

6:10 p.m. – 6:30 p.m. **Distinguished Andrologist Award**

6:30 p.m. – 7:30 p.m. **ASA KEYNOTE LECTURE**
Epigenetic Regulation of Mammalian Development: Implications for Stem Cell Therapy and Cloning
Rudolf Jaenisch, MD
Whitehead Institute, Massachusetts Institute of Technology
(Introduced by Christina Wang, MD)

7:30 p.m. – 9:30 p.m. **Welcome Reception**
Location: City Center

SUNDAY, APRIL 22, 2007

6:30 a.m. – 8:00 a.m. **Past President's Breakfast**
Location: Buccaneer A

7:00 a.m. – 4:00 p.m. **Exhibit Hall Open**
Location: Regency Ballroom 1-3

7:00 a.m. – 6:00 p.m. **Registration**
Location: Registration Counter, Ballroom Level

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
Use of Proteomic and Genomic Signatures for Prostate Cancer Screening, Diagnosis and Treatment
Arul M. Chinnaiyan, MD, PhD
University of Michigan Medical Center
(Introduced by Dana Ohl, MD)

9:00 a.m. – 9:15 a.m. **Distinguished Service Award**

9:15 a.m. – 10:45 a.m. **SYMPOSIUM I – UPDATE ON EPIDIDYMAL AND SPERM FUNCTION: TRANSPORTERS, CHANNELS AND SENSORY RECEPTORS**
Co-chairs: E. Mitch Eddy, PhD and Kim Chau, BS

Acid-base Transporters in the Male Reproductive Tract: Potential Consequences of Malfunction
Sylvie Breton, PhD
Massachusetts General Hospital

Ion Channels and Sperm Function: Potential Clinical Implications
Timothy Quill, PhD
University of Texas-Southwestern

Odorant Receptor and Sperm Function: Do Sperm Have a Sense of Smell?
Marc Spehr, PhD
Ruhr-Universitaet Bochum

10:45 a.m. – 12:30 p.m. **Poster Session I**
Location: Regency Ballroom 1-3

12:30 p.m. – 2:00 p.m. **Lunch (on your own)**

*Locations subjects to change

schedule at a glance

12:30 p.m. – 2:00 p.m.
Women in Andrology Luncheon and Discussion
(not included in registration fee; tickets required)
Location: Buccaneer B

2:00 p.m. – 3:30 p.m.
Concurrent Oral Sessions I and II

Spermatogenesis, Infertility, Contraception and Sexual Function
Moderators: John K. Amory, MD and Michaela Luconi, PhD
Location: Regency 4-7

Sperm Function, ART, Epididymis, and Fertilization
Moderators: Patricia A. Martin-DeLeon, PhD and Genevieve Griffiths, BA
Location: Garrison Suite

3:30 p.m. – 4:00 p.m.
Refreshment Break
Location: Regency Foyer

4:00 p.m. – 4:45 p.m.
LECTURE I
Development and Potential Uses of Selective Androgen Receptor Modulators (SARM's)
James T. Dalton, PhD
Ohio State University
(Introduced by Glenn R. Cunningham, MD)

4:45 p.m. – 5:30 p.m.
LECTURE II:
Preimplantation Genetics: The Technology, The Medicine and The Bioethics
Masaru Okabe, PhD
Osaka University
(Introduced by Janice L. Bailey, PhD)

6:00 p.m. – 8:00 p.m.
Trainee Forum and Mixer
(All Trainee Travel Awards will be distributed and celebrated at this event)
Location: Buccaneer A

MONDAY, APRIL 23, 2007

7:00 a.m. – 12:30 p.m.
Exhibit Hall Open
Location: Regency Ballroom 1-3

7:00 a.m. – 6:00 p.m.
Registration
Location: Registration Counter, Ballroom Level

8:00 a.m. – 9:00 a.m.
WOMEN IN ANDROLOGY LECTURE
Human Germ Cell Differentiation: Clinical Implications for Male Infertility
Renee A. Reijo Pera, PhD
University of California at San Francisco
(Introduced by Mary M. Lee, MD)

9:00 a.m. – 9:15 a.m.
Young Andrologist Award

9:15 a.m. – 10:45 a.m.
SYMPOSIUM II – CLINICAL UROLOGY UPDATE: TOO LONG, TOO CURVED AND TOO QUICK
Co-chairs: Wayne J.G. Hellstrom, MD and Peter Liu, MBBS, PhD

Basic Insights into the Pathophysiology of Priapism: Enough Already
Arthur L. Burnett, MD
Johns Hopkins University

Molecular and Cellular Basis of Peyronie Disease: Mr. Snappy of the Penis
Jacob Rajfer, MD
UCLA Medical Center

Advances in the Pathophysiology and Pharmacotherapy of Premature Ejaculation: Slow Down You're Moving Too Fast
Ridwan Shabsigh, MD
Columbia University

10:45 a.m. – 12:30 p.m.
Poster Session II
Location: Regency 1-3

12:30 p.m. – 2:00 p.m.
Lunch (on your own)

12:30 p.m. – 2:00 p.m.
MENTORING LUNCHEON SPONSORED BY THE DIVERSITY, TRAINEE AFFAIRS AND ETHICS COMMITTEES
Ethics in Andrology
Stanley G. Korenman, MD
David Geffen School of Medicine at UCLA
Location: City Center
(not included in registration; tickets required)

12:30 p.m. – 2:00 p.m.
Editorial Board Luncheon
Location: Harborview - 16th Floor

2:00 p.m. – 3:30 p.m.
SYMPOSIUM III – SPERMATOGENESIS, MALE INFERTILITY AND REPRODUCTIVE TOXICOLOGY
Co-chairs: Kenneth P. Roberts, PhD and Sarika Saraswati, MS

Role of Protein Kinase A in Spermatogenesis and Sperm Function: Of Mice and Men
G. Stanley McKnight, PhD
University of Washington

DNA Methylation: Implications for Genomic Imprinting, Development and Male Infertility
Jacquetta Trasler, MD, PhD
McGill University, Montreal, Canada

schedule at a glance

Novel Approaches for Conducting Environmental Epidemiology Studies on Male Reproductive Health
Sally Perreault Darney, PhD
US Environmental Protection Agency

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Regency Foyer

4:00 p.m. – 4:45 p.m. **LECTURE III (European Academy of Andrology Lecture)**
The European Male Aging Study: What Happens as Men Age and What Are the Implications?
Location: Regency Ballroom 1-3
Frederick C.W. Wu, MD
University of Manchester, United Kingdom
(Introduced by William J. Bremner, MD, PhD)

4:45 p.m. – 5:30 p.m. **LECTURE IV**
Location: Regency Ballroom 1-3
Testicular Macrophages: Friends or Enemies Within
James C. Hutson, PhD
Texas Tech University
(Introduced by Kate Loveland, PhD)

5:30 p.m. **ASA Business Meeting**
Location: Regency Ballroom 1-3
Outstanding Trainee Investigator and Trainee Awards

7:30 p.m. – 10:30 p.m. **Annual Banquet**
(not included in registration fee; tickets required)
Location: Starship Cruise (Boat sails at 8:00 p.m.)

TUESDAY, APRIL 24, 2007

7:00 a.m. – 8:00 a.m. **2008 Program Committee Meeting**
Location: Channelside 1

7:30 a.m. – 11:00 a.m. **Registration**
Location: Registration Counter, Ballroom Level

8:00 a.m. – 9:30 a.m. **SYMPOSIUM IV – REGULATION OF PROSTATE CANCER GROWTH**
Location: Regency Ballroom 1-3
Co-Chairs: Gail S. Prins, PhD and Stephanie Page, MD, PhD

Regulation of Apoptosis and Prostate Cancer Growth
Natasha Kyprianou, MD, PhD
University of Kentucky Medical Center

Androgen Receptor and Control of Prostate Cancer Growth
Norman Greenberg, PhD
Fred Hutchinson Cancer Research Center, Seattle, Washington

Vitamin D Regulation of Prostate Cancer Growth: Clinical Implications
Kerry L. Burnstein, PhD
University of Miami School of Medicine

9:30 a.m. – 9:45 a.m. **Break**
Location: Regency Foyer

9:45 a.m. – 10:45 a.m. **INTERNATIONAL LECTURE**
Location: Regency Ballroom 1-3
Clinical Relevance of Androgen Receptor Polymorphism
Eberhard Nieschlag, MD
University of Muenster, Germany
(Introduced by Patricia S. Cuasnicu, PhD)

10:45 a.m. – 12:15 p.m. **SYMPOSIUM V – INTRAMURAL AND EXTRAMURAL TESTIS CELL BIOLOGY**
Location: Regency Ballroom 1-3
Co-chairs: Don F. Cameron, PhD and Monica Schwarcz, MD

Extra-Testicular Sertoli Cell: Immunobiology and Role in Transplantaion
Jannette Dufour, PhD
Texas Tech University

Role of Steroidogenic Enzymes in Germ Cells on Sperm Function and Fertility
Vassilios Papadopoulos, PhD
Georgetown University

Spermatogonial Stem Cells in Primate Testes: Potential Therapeutic Uses
Stefan Schlatt, PhD
University of Pittsburgh School of Medicine

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president's welcome

Christina Wang, MD

Welcome to the 32nd Annual Meeting of the American Society of Andrology and to Tampa for an exciting three days of scientific interchange, collaboration and friendship. Al Matsumoto and his Program Committee have arranged an exciting program with the theme "On the Cutting Edge of Basic, Translational and Clinical Andrology: Implications on the Future of Reproductive Health."

The ASA Annual meeting opens on Saturday evening with the ASA Lecture "Epigenetic Regulation of Mammalian Development: Implications for Stem Cell Therapy and Cloning" presented by Rudolf Jaenisch. The Women in Andrology Lecture follows the same theme: "Human Germ Cell Differentiation: Clinical Implications for Male Infertility" to be given by Renee Reijo Pera and followed later in the meeting by "Molecular Basis of Fertilization: Clinical Implications of Izumo and Beyond" by Masaru Okabe. The AUA lecturer in 2007 is Arul Chinnaiyan on "Use of Proteomic and Genomic Signatures for Prostate Cancer Screening and Diagnosis" accompanied by a symposium on "Regulation of Prostate Cancer Growth." The ASA 2007 program features our Distinguished Andrologist, Ebo Nieschlag speaking on "Clinical Relevance of Androgen Receptor Polymorphism" as well as Fred Wu as the International Lecturer (the European Academy of Andrology lecturer) on "The European Male Aging Study: What Happens When Men Age and What are the Implications?" and James Dalton on "Development and Potential Uses of Selective Androgen Receptor Modulators," our next generation of androgens. New for ASA is a discussion on "Ethics in Andrology" by Stanley Korenman at the lunch session on Monday, which is jointly sponsored by the Trainee Affairs, Diversity and Ethics Committee.

Other presentations highlight diverse aspects of andrology reflecting the interests of our members: testicular macrophages, sperm transporters, channels and sensory receptors, reproductive toxicology and infertility, male sexual dysfunction, and intramural and extramural testis cell biology. Throughout the meeting, please take your time to attend oral abstract and poster presentations, discuss findings with their authors, meet and encourage our young investigators to pursue their career in Andrology and visit the expanded exhibits.

Immediately preceding the meeting are the XIX North American Testis Workshop focusing on "Chromosome Structure and Gene Expression," the Andrology Laboratory Workshop, which consists of two hands-on sections. "Sperm Morphology" and "Quality Control," and the satellite symposium in the afternoon of April 21 on "Androgen Deficiency in Men: Challenges and Solutions."

There is plenty of opportunity to meet informally with your colleagues and trainees at the many events during the meeting, including the Laboratory Science Forum, Women in Andrology Luncheon, and the Trainee Forum and Mixer. Don Cameron and his team have been working very hard to ensure we will enjoy the sun, beach, waterside cafes and the banquet on a boat across Tampa Bay on Monday. Enjoy the 2007 ASA Annual Meeting.

Christina Wang, MD
President, American Society of Andrology

Past Presidents of the American Society of Andrology

1975-1977	Emil Steinberger	1991-1992	David W. Hamilton
1977-1978	Don W. Fawcett	1992-1993	Ronald S. Swerdloff
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1979-1980	Nancy J. Alexander	1994-1995	Glenn R. Cunningham
1980-1981	Philip Troen	1995-1996	Marie-Claire Orgebin-Crist
1981-1982	Richard M. Harrison	1996-1997	Arnold M. Belker
1982-1983	Richard J. Sherins	1997-1998	Terry T. Turner
1983-1984	Andrzej Bartke	1998-1999	Richard V. Clark
1984-1985	Rudi Ansbacher	1999-2000	Barry T. Hinton
1985-1986	Anna Steinberger	2000-2001	J. Lisa Tenover
1986-1987	William D. Odell	2001-2002	Barry R. Zirkin
1987-1988	Larry L. Ewing*	2002-2003	Jon L. Pryor
1988-1989	C. Wayne Bardin	2003-2004	Gail S. Prins
1989-1990	Rupert Amann	2004-2005	William J. Bremner
1990-1991	Howard Nankin	2005-2006	Sally Perreault Damey
		*Deceased	

American Society of Andrology

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Journal of Andrology	Matthew P. Hardy, PhD; New York, NY (Co-Editor)
	Peter N. Schlegel, MD; New York City, NY (Co-Editor)
Laboratory Science Forum	David S. Karabinus, PhD, HCLD; Fairfax, VA
Liaison Committee	Joel L. Marmar, MD; Camden, NJ
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Program Committee	Alvin M. Matsumoto, MD; Seattle, WA
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Publications Committee	Marvin L. Meistrich, PhD; Houston, TX (Co-Chair)
Testis Workshop	E. Mitch Eddy, PhD; Research Triangle Park, NC (Co-Chair)
	Michael D. Griswold, PhD; Pullman, WA (Co-Chair)
Trainee Affairs	Michael A. Palladino, PhD; West Long Branch, NJ

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NOTICE TO READERS

Every effort has been made to ensure that the information printed here is correct; however, details are subject to change.

general information

about tampabayflorida

Tampa Bay's lush natural landscape, multi-cultural makeup and endless assortment of in-and-outdoor activities make it a paradise for outdoorsmen and spectators alike.

Tampa is home to more than 26 miles of sun-kissed white sugar sand beaches on the Gulf of Mexico, making it a favored place to sunbathe and a picturesque destination. Tampa also features dozens of water sports for every preference, whether that be sport-fishing, relaxing on a sailboat, or kayaking. The more adventurous can also take in the spectacular views of the Florida Coast from below the water by snorkeling or diving.

Busch Gardens, Tampa's premier theme park, offers exciting encounters with replicated and real aspects of the African continent. In addition to the thrilling rides, live shows, restaurants, games and more, the park is also home to one of the country's premier zoos, featuring more than 2,000 animals. The Florida Aquarium, another Tampa attraction, tells Florida's water story from a drop of water to undersea life, and features more than 10,000 aquatic plants and animals both native to Florida and from all over Once done sightseeing, grab a bite at any one of Florida's culinary favorites, boasting fresh seafood creations from multi-national chefs. Tampa's steamy nightlife benefits from a multi-national background and all genres of music can be heard flowing from the city's many nightclubs.

Weather

In April, the temperature ranges from an average low of 62 F to an average high of 81 F.

hotelinformation

Hyatt Regency Tampa
Two Tampa Center
Tampa, Florida 33602
Phone: 813-222-4970
Fax: 813-204-3095

Rate: $189.00 Single/Double Occupancy
$199.00 Triple/Quad Occupancy

Room rates are exclusive of applicable state and local taxes (which are currently 12%) or applicable service, or hotel specific fees in effect at the Hotel at the time of the meeting.

All reservations must be made individually through the Hotel's Reservation Department by calling (813) 225-1234 or 800-233-1234 for Hyatt Hotels and Resorts Central Reservations. Be sure to mention that you are with the ASA Annual meeting for the discounted rate. Reservations should be made prior to *March 25, 2007* to receive the American Society Andrology

travelandtransporationinformation

Air Travel

United Airlines is the official airline company for the ASA Annual Meeting. You are not required to use them, but we encourage you to take advantage of their special offer.

United will provide to passengers traveling to attend the Meeting defined above round-trip transportation on scheduled service to the Meeting City on United, United Express, TED by United or United code share flights operated by US Airways, US Airways Express and Air Canada for travel in or between the United States, Canada, Caribbean, San Juan< US Virgin Islands and Mexico Beaches at fares at either (1) a 2% discount off Q,V,W,S, T and L published fares in effect when the tickets are purchased subject to restrictions and rules applicable to the fare purchased, or (2) a 5% discount off applicable M, E, U, H fares in effect when tickets are purchased subject to all restrictions and rules applicable to the fare purchased, or (3) a 10% discount off applicable F, A, P, C, D, Y and B fares in effect when tickets are purchased purchased **more than 30 days** subject to all restrictions and rules applicable to the fare purchased. For tickets **prior** to travel within the contracted Travel Period the following discounts apply: 7% discount off Q, V, W, S, T, and L published fares, or 10% discount off applicable M, E, U, H fares or at 15% discount off applicable F, A, P, C, A, D, Y and B fares.

Reservations, schedule and ticketing information may be obtained by calling the Meeting Plus Reservation Center at **(800) 521-4041** and referring to your Meeting Plus ID Code, **563ON**.

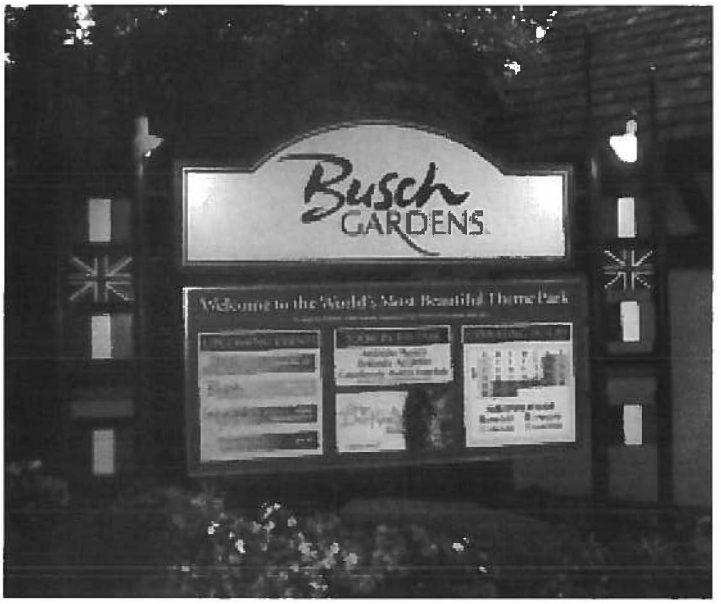
Ground Transportation

Car Rental

Avis Rent A Car is the official rental car company for the ASA Annual Meeting. You are not required to use them, but we encourage you to take advantage of their special offer.

You must return the car at the same renting location, or additional surcharges may apply. All rates include unlimited free mileage. Rates do not include any state or local surcharges, tax, optional coverage or gas refueling charges. Weekend daily rates are available from 12:00 p.m. Thursday through 11:59 p.m. Monday. When making your reservations, dial **(800) 331-1600** and mention code **"J901055"** to receive the discounted rates.

http://www.avis.com/AvisWeb/html/meetings/go2.html?AWD=J901055&NAME=American+Society+of+Androlog y&FDATE=04182007&TDATE=04252007&LOCATION=Tampa,+Florida&EVENT=0



events & activities

Lab Science Forum and Luncheon:
Saturday, April 21, 2007
11:45 p.m. – 1:15 p.m.
Location: Esplanade
Join us for the 2007 Lab Science Forum on Luncheon. The luncheon will include a talk entitled "Sperm Motility Enhancers, Do They Really Improve Outcomes?" by William E. Roudebush, PhD, HCLD (ABB) from Beckman Coulter, Inc.
Cost: $30.00 per person. Please sign up for this event on the registration form. (Included in Andrology Lab workshop fee)

Welcome Reception
Saturday, April 21, 2007
7:30 p.m. – 9:30 p.m.
Location: Regency Ballroom
Join us on Saturday, April 21, 2007, at 7:30 p.m. for a Welcome Reception, to connect with friends and colleagues. Admission to the reception is included in your ASA registration fee (not included if you only register for the Testis Workshop, Andrology Lab Workshop and/or Special Symposium).
Cost: One ticket to the reception along with two drink tickets are included in your ASA registration fee; additional tickets may be purchased for $30.00 each.
Business casual or casual attire is appropriate.

Women in Andrology Luncheon and Roundtable Discussions
Sunday, April 22, 2007
12:30 p.m. – 2:00 p.m.
Location: Bucaneer B
The Women in Andrology group, which promotes the visibility and contributions of women within the Society and the field of andrology, meets for lunch each year at the ASA's Annual Meeting. This year's luncheon, to be held Sunday, April 22, will provide an opportunity for female andrologists to 'meet and greet,' to share recent accomplishments and future plans, hear about changes over the years in the role of women in andrology and science, and gain insights on key issues in career development.
Cost: $20.00 per person. Please sign up for this event on the registration form.
Business casual or casual attire is appropriate.

Trainee Forum and Mixer
Sunday, April 22, 2007
6:00 p.m. – 8:00 p.m.
Location: Bucaneer A
The ASA Trainee Forum and Mixer provide the opportunity for Trainee members to meet other trainees as well as meet with more established members of the Society. This is a relaxed, informal event with appetizers, beer, and wine provided. Senior members of the Society will be present for an informal "forum and discussion group" setting to answer your questions about relevant topics such as grant-writing, searching for a post-doc or job, alternative PhD career paths, succeeding in the clinic or lab, etc. All members of the Society are welcome. Please check the appropriate box on the registration form if you will be attending.
Business casual or casual attire is appropriate.

Mentoring Luncheon Sponsored by the Diversity, Trainee Affairs and Ethics Committees
"Career Development"
Monday, April 23, 2007
12:30 p.m. – 2:00 p.m.
Location: City Center
The Diversity, Trainee Affairs and Ethics Committees are co-sponsoring a mentoring luncheon on Monday, April 23, 2007. This event is designed to provide career development and networking opportunities for ASA meeting attendees, particularly trainees and minorities. Dr. Korenman will introduce for discussion a number of ethical questions that arise for andrologists in practice. They include the justification of treatment with androgens in men and women, the relationship to prostate cancer, the ethical limitations of practice guidelines in their relationship to correct practice and individualized practice.
Cost: $5.00 for trainees; $10.00 for non-trainees. Please sign up for this event on the registration form.

Annual Banquet
Monday, April 23, 2007
7:30 p.m. – 10:30 p.m.
Location: Starship Dock
There's no better place to experience Tampa Bay's beauty and charm than aboard the newly expanded Yacht StarShip. Yacht StarShip's 180-foot, $8 million super yacht features star-quality service, out-of-this-world views, dazzling live entertainment and four-star cuisine freshly prepared by our Executive Chef and galley staff, and is recognized by AAA with a three diamond rating, making us the first dining yacht in the United States to be honored with this distinction.
Dress: Business Casual
Cost: $70.00 per person. $35.00 per person for trainees. Please sign up for this event on the registration form.

Program Updates

Obtain detailed meeting information, view updates and register online at **www.andrologysociety.org**.

Program Committee

Alvin M. Matsumoto, MD; Seattle, WA (Chair)
John RobertAitken, ScD, FRSE; Callaghan, NSW AUSTRALIA
Aniela Bollendorf, MT; Melrose Park, PA
Arthur L. Burnett, MD; Baltimore, MD
Nina Sarah Davis, MD; Portland, OR
Ina Dobrinski, DVM, PhD; Kennett Square, PA
E. Mitch Eddy, PhD; Research Triangle Park, NC
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Shuk-Mei Ho, PhD; Cincinnati, OH
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Sally Perreault Darney, PhD; Research Triangle Park, NC
Jacquetta M. Trasler, MD, PhD; Dorval, QUEBEC CANADA
Paul Jacob Turek, MD; San Francisco, CA
Terry T. Turner, PhD; Charlottesville, VA
Christina Wang, MD; Torrance, CA

message from the program chair

Alvin M. Matsumoto, MD

On behalf of the 2007 Program Committee, I welcome you to Tampa and the 32nd Annual Meeting of the American Society of Andrology (ASA). For the next three days, we hope that you will learn something, have lively scientific exchange, formulate new ideas, form new collaborations and friendships and reconnect with old colleagues and friends. For those of you who are not members of the ASA, I encourage you to join our unique scientific community.

I thank all members of the 2007 Program Committee: (R. John Aitken, ScD; Aniela Bollendorf, MT; Arthur L. Burnett II, MD; Nina S. Davis, MD; Ina Dobrinski, DVM, PhD; E. Mitch Eddy, PhD; Janice P. Evans, PhD; Matthew P. Hardy, PhD; Shuk-Mei Ho, PhD; Gary Klinefelter, PhD; Delores Lamb, PhD; Mary M. Lee, MD; Kate Loveland, PhD; Michael G. O'Rand, PhD; Jacquetta M. Trasler, MD, PhD; Paul J. Turek, MD; and Terry T. Turner, PhD) for their hard work and commitment to the ASA, Robert Oates (2006 Program Chair) and Christina Wang (ASA President and 2005 Program Chair) for their help and guidance, and all members of the ASA who suggested topics and speakers for the program.

The ASA Meeting is unique in bringing together the diverse research, laboratory and clinical disciplines in the field of male reproduction. The Program Committee has worked hard to put together a broad-based program that reflects the Society's diversity and its members' interests. The title, "On the Cutting Edge of Basic, Translational and Clinical Andrology," encapsulates the essence of the meeting. The lectures and symposia presentations will be given by leaders in the field and will cover state-of-the-art developments in the manifold areas of male reproductive biology and highlight the translational aspects of research in these areas.

The meeting will open with the ASA Keynote Lecture by Dr. Rudolf Jaenisch of the Whitehead Institute, Massachusetts Institute of Technology, who will speak on pioneering work performed in his laboratory over the last three decades in a lecture entitled, "Epigenetic Regulation of Mammalian Development: Implications for Stem Cell Therapy and Cloning." The AUA Lecture will be presented by Arul M. Chinnaiyan of the University of Michigan Medical Center, who will speak on "Use of Proteomic and Genomic Signatures for Prostate Cancer Screening, Diagnosis and Treatment," a burgeoning area in clinical and molecular medicine. The Women in Andrology Lecture will be given by ASA member, Dr. Renee A. Reijo Pera of the University of California at San Francisco, who will present a lecture entitled, "Human Germ Cell Differentiation: Clinical Implications for Male Infertility." The ASA International Lecture will be given by a long-standing ASA member and this year's Distinguished Andrologist, Dr. Eberhard Nieschlag of the University of Munster in Germany, who will speak on the "Clinical Relevance of Androgen Receptor Polymorphism."

Other exciting and stimulating lectures by international experts in their fields include: "Development and Potential Uses of Selective Androgen Receptor Modulators (SARMs)," by Dr. James T. Dalton, Ohio State University; "Preimplantation Genetics: The Technology, The Medicine and The Bioethics," by Dr. Masaru Okabe, Osaka University, Japan;" The European Male Aging Study: What Happens as Men Age and What Are the Implications?" The European Academy of Andrology Lecture, given by ASA Member, Dr. Frederick C.W. Wu, University of Manchester, UK; "Testicular Macrophages: Friends or Enemies Within," by Dr. James C. Hutson, Texas Tech University; and "Ethics in Andrology," sponsored by the ASA Diversity, Trainee Affairs and Ethics Committees, given by Dr. Stanley Korenman, David Geffen School of Medicine at UCLA.

There will be five state-of-the art symposia: "Update on Epididymal and Sperm Function: Transporters, Channels and Sensory Receptors," featuring ASA member, Dr. Sylvie Breton, Massachusetts General Hospital and Harvard Medical School, Dr. Timothy Quill, University of Texas Southwestern Medical Center, and Dr. Marc Spehr, Ruhr-Universitaet-Bochum;" Clinical Urology Update: Too Long, Too Curved and Too Quick," featuring ASA member, Dr. Arthur L. Burnett, Johns Hopkins University, Dr. Jacob Rajfer, UCLA Medical Center, and Dr. Ridwan Shabsigh, Columbia University; "Spermatogenesis, Male Infertility and Reproductive Toxicology," featuring Dr. G. Stanley McKnight, University of Washington, ASA member, Dr. Jacquetta Trasler, McGill University, and ASA Past-President, Dr. Sally Perreault Darney, US Environmental Protection Agency; "Regulation of Prostate Cancer Growth," featuring Dr. Natasha Kryprianou, University of Kentucky, Dr. Norman Greenberg, Fred Hutchinson Cancer Research Center, and Dr. Kerry L. Burnstein, University of Miami School of Medicine; and "Intramural and Extramural Testis Cell Biology," featuring ASA members, Dr. Janette Dufour, Texas Tech University, Dr. Vassillios Papadopoulos, Georgetown University, and Dr. Stefan Schlatt, University of Pittsburgh School of Medicine.

There will be two oral sessions, held concurrently, and two poster sessions, held on separate days that will feature diverse and interesting initial reports by ASA members. There should be plenty of time to interact with the presenters and ASA members with mutual research interests during these sessions.

The 2007 ASA Program Committee worked with the Chair, Michael D. Griswold, Washington State University, the Co-Chair, E. Mitch Eddy, National Institute of Environmental Health Sciences, and the Program Committee of the XIXth Testis Workshop, entitled "Chromosome Structure and Gene Expression," to provide non-overlapping and complementary programs.

The 2007 ASA Andrology Laboratory Workshop and Laboratory Science Forum, organized by Dr. Aniela Bollendorf, will be held after the Testis Workshop program concludes. The Andrology Laboratory Workshop program will focus on "Sperm Morphology – A Hands On Workshop," featuring ASA members Dr. Susan Rothman and Angela Reese, Fertility Solutions, Inc., Dr. David Karabinus, Genetics and IVF Institute, and Dr. Bollendorf, and the Laboratory Science Forum Luncheon will present a program entitled, "Quality Control –A Hands On Workshop," featuring Dr. Steve Simon, Children's Mercy Hospital in Kansas City and ASA member Steven Schrader, National Institute of Occupational Health and Safety. Taking place concurrently with the Andrology Laboratory Workshop will be a Special ASA Continuing Medical Education Symposium, entitled, "Androgen Deficiency in Men: Challenges and Solutions," organized by ASA President Christina Wang. This program will feature ASA members, Dr. Frances Hayes, Massachusetts General Hospital and Harvard Medical School, Dr. Ronald Swerdloff, David Geffen School of Medicine at UCLA Harbor, Dr. Glenn Cunningham, Baylor College of Medicine, and Dr. Ridwan Shabsigh, Columbia University.

This exciting program is made possible by support from the National Institute of Child Health and Human Development of the National Institutes of Health, unrestricted educational grants from our industry partners who are acknowledged by name later in this program book, and all members of the ASA. On behalf of the ASA, I thank all sponsors for their continued generous support of our Society and meeting. Finally, I thank all those who work in the ASA Executive Office at WJ Weiser and Associates, especially Debbie Roller, Ann Marie Bray and Laura Sanchez, for their assistance in formulating, organizing and finalizing all elements of the Annual Meeting program.

asa lecturer award

Rudolf Jaenisch, MD

Rudolf Jaenisch received his MD from the University of Munich in 1967. Since 1984 he has been a Founding Member of the Whitehead Institute for Biomedical Research and a Professor of Biology at the Massachusetts Institute of Technology, and in 2005 he established the Human Stem Cell Facility at the Whitehead.

Dr. Jaenisch is a pioneer in making transgenic mice, some of which have produced important advances in understanding cancer, neurological and connective tissue diseases, and developmental abnormalities. These methods have been used to explore basic questions such as the role of DNA modification, genomic imprinting, X chromosome inactivation, nuclear cloning, and, most recently, the nature of stem cells. The Jaenisch laboratory is known for its expertise in cloning mice and in studying the many factors that contribute to the success and failure of that process. They have more recently gained insights into therapeutic cloning, and have indeed rescued mice having a genetic defect through therapeutic cloning and gene therapy. In addition, using mice as a model and a technique called "altered nuclear transfer," the Jaenisch lab has recently demonstrated that it is possible to procure embryonic stem cells without harming a viable embryo.

Dr. Jaenisch has received a number of awards, including election as a Fellow in the American Academy of Arts and Sciences in 1992, the Boehringer Mannheim Molecular Bioanalytics Prize in 1996, the First Peter Gruber Foundation Award in Genetics in 2001, the Robert Koch Prize for Excellence in Scientific Achievement in 2002, election as a Member of International Society for Stem Cell Research in 2002, election as a Member of National Academy of Sciences in 2003, the Charles Rodolphe Brupracher Foundation Cancer Award in 2005 and the Max Delbrück Medal in Molecular Medicine in 2006.

Serono Lectureship Recipients

1980	C. Alvin Paulsen
1981	Pierre Soupart
1982	Kevin J. Catt & Maria L. Dufau
1983	J. Michael Bedford
1984	C. Wayne Bardin
1985	David M. De Kretser
1986	Ronald S. Swerdloff
1987	Roger V. Short
1988	Roger Guillemin
1989	Frank S. French
1990	David C. Page
1991	Tony M. Plant
1992	Yves Clermont
1993	Leroy Hood
1994	Michael D. Griswold
1995	Marie-Claire Orgebin-Crist
1996	Norman B. Hecht
1997	Patrick C. Walsh
1998	Jurrien Dean
1999	Neal First
2000	Bert O'Malley
2001	John D. Gearhart
2002	David Botstein
2003	Victor D. Vacquier

ASA Lectureship Recipients

2004	Judith Kimble
2005	David Page
2006	John R. Aitken

distinguished andrologist award

Eberhard (Ebo) Nieschlag

The Distinguished Andrologist Award is the highest award bestowed by the American Society of Andrology and is presented annually to a senior investigator who has made outstanding contributions to the progress of Andrology. The American Society of Andrology is pleased to recognize Dr. Eberhard (Ebo) Nieschlag of the University of Muenster in Muenster, Germany as the 2007 Distinguished Andrologist for his outstanding clinical and basic science contributions to our understanding of male reproductive function, as well as his exceptional record of leadership and service in many facets of male reproductive biology.

Dr. Nieschlag was born and grew up in Bad Godesberg, Germany, and he attended medical school at the Universities of Bonn and Munich, graduating in 1967 with his medical degree. He then received additional clinical training, as well as research experience, at University College London and at the Medical Research Council in Edinburgh, Scotland before joining the Department of Clinical Endocrinology at the University of Mainz in Germany from 1968 to 1970. He worked at NIH as a clinical fellow under the direction of Dr. Mort Lipsett from 1971-1972, and then he joined the Department of Internal Medicine at the University of Dusseldorf in 1972. He subsequently moved to the University of Munster, where he served as the Director of the Department for Experimental Endocrinology from 1976-1986, and he also served as the Director of the Max Planck Clinical Research Unit for Reproductive Medicine at the University of Munster from 1980-1988. He has held his present position as the Director of the Institute for Reproductive Medicine at the University of Munster since 1986, and since 1992, he has also been a Full Professor of Reproductive Medicine.

Dr. Nieschlag has distinguished himself as a scientist, clinician, teacher and leader in andrology. He has made contributions in many areas of clinical andrology, where he has had a special focus on the areas of the aging male, male hypogonadism and infertility, testosterone treatment and male contraception. However, he has been equally prolific and influential in terms of his basic science contributions to the field, and his contributions to the field span almost every area of andrology. Dr. Nieschlag's published contributions to medicine and andrology have been prodigious; he has authored over 700 journal articles, 200 book chapters and 10 books. He has trained over 140 researchers, approximately half from outside Europe, and these former students and trainees have gone on successful careers in medical centers, universities and research institutes throughout the world.

Dr. Nieschlag has made major administrative and service contributions to Andrology and the broader scientific community during his career. He was the first President of the International Society of Andrology, President of the European Academy of Andrology, and Chairman of the Steering Committee of the Task Force on the Regulation of Male Fertility at the World Health Organization (1985-1990). He has been the leader of the Male Summit conferences on Male Contraception, bringing investigators, granting agencies (e.g., NIH, Conrad, Population Council) and industry together for annual meetings devoted to sharing information and designing collaborations and multinational studies. Dr. Nieschlag also has been a long-term member of the International Committee on Contraceptive Research (ICCR) and the Interdisciplinary Committee on Guidelines for ART of the German Federal Medical Board since 1985. Professor Nieschlag also established a German external quality control program for spermatology that is utilized by a large number of participating laboratories (160 in 2006).

Dr. Nieschlag's extensive accomplishments have garnered him many honors and awards, including the Ernst Jung Prize for Medicine in 1996, election to fellowship in the Royal College of Physicians (London) in 2005, the Premio di Andrologia, J.M. Pomerol, Fundacio Puigvert (Barcelona) in 2005, and an honorary doctorate from the Medical University in Lodz (Poland) in 2006, to name just a few. The ASA is proud to honor Dr. Nieschlag's broad and outstanding accomplishments in Andrology by selecting him as our Distinguished Andrologist for 2007.

Distinguished Andrologists

1976	Roy O. Greep & M.C. Chang
1977	Robert E. Mancini
1978	Robert S. Hotchkiss
1979	Thaddeus Mann
1980	John MacLeod
1981	Alexander Albert
1982	Eugenia Rosemberg
1983	Kristen B.D. Eik-Nes
1984	Mortimer B. Lipsett
1985	Robert H. Foote
1986	Alfred D. Jost
1987	Emil Steinberger
1988	Yves W. Clermont
1989	C. Alvin Paulsen
1990	Marie-Claire Orgebin-Crist
1991	Philip Troen
1992	C. Wayne Bardin
1993	Anna Steinberger
1994	Richard J. Sherins
1995	Rupert P. Amann
1996	J. Michael Bedford
1997	Brian P. Setchell
1998	Ryuzo Yanagimachi
1999	Richard D. Amelar
2000	Bayard T. Storey
2001	Frank S. French
2002	Geoffrey M. H. Waites
2003	David M. de Kretser
2004	Ronald Swerdloff
2005	Mitch Eddy
2006	Norman Hecht

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

distinguished service award

Sally Perreault Darney,

The Distinguished Service Award recognizes an individual for their service contributions to the American Society of Andrology. This year's recipient is Dr. Sally Perreault Darney, who is presently the Acting Director for the Reproductive Toxicology Division of the U.S. Environmental Protection Agency in Research Triangle Park, NC. Dr. Darney, a native of White Plains, NY, received her BA degree from Newton College, in Newton, MA, and her MAT degree from Brown University in Providence, RI. After several years of teaching biology at Bryant College and Community College of Rhode Island, she resumed graduate studies, earning her PhD from the University of Hawaii in 1980. Following the completion of her doctoral degree, she returned to the East Coast to pursue postdoctoral work with Dr. Barry Zirkin at Johns Hopkins University in Baltimore. She subsequently accepted a position in 1984 in the Reproductive Toxicology Division of the U.S. Environmental Protection Agency, where she has risen through the ranks to serve as Chief of several different divisions before assuming her current position as Acting Director of the Reproductive Toxicology Division in 2004.

Dr. Darney has been a member of the American Society of Andrology since 1980, and she has served the ASA in a wide variety of capacities. She was President-Elect during 2004-2005, and recently served as President for 2005-2006, completing her term at the Annual Meeting last spring. She is now serving as Past President for 2006-2007. Dr. Darney has been a member of the Executive Council and has chaired a number of committees including Program, Nominations, Awards, Local Arrangements, and Postgraduate Course. She has also worked on a variety of other committees within ASA, including Membership, Future Meetings, Development and Publications and has chaired the Women in Andrology Group. She has served on the Editorial Board of the Journal of Andrology and will be one of three new co-editors starting in July 2007. In addition to her extensive work on behalf of ASA, and she has also had significant leadership roles in other scientific societies on committees and on editorial boards.

Dr. Darney's exemplary record of service to ASA was accomplished as she undertook increasing managerial and science leadership responsibilities at EPA. In addition, she has forged a strong international research reputation in the areas of reproductive toxicology and mammalian fertilization, where her interests have included toxicant-induced impairment of gamete production/function and its impact on fertility and early pregnancy loss; genetic markers in sperm; critical windows of exposure for gamete development; and regulation of molecular events associated with reactivation of the sperm nucleus and early gene expression following fertilization.

In summary, Dr. Darney's service contributions to ASA and other societies and to the broader scientific community have been extensive, and remind us that the success of societies such as ours depends on the commitment of the members and their willingness to give their time and effort to move the society forward. Her long history of volunteerism and leadership at all levels of ASA make her a most deserving recipient of the Distinguished Service Award. The ASA gratefully recognizes her extensive and tireless work on behalf of the society with this award, and looks forward to her continued contributions to ASA and male reproductive biology for many years to come.

Distinguished Service Award Recipients

1994	C. Alvin Paulsen
1995	Andrzej Bartke
1996	Philip Troen
1997	Marie-Claire Orgebin-Crist
1998	Rupert P. Amann
1999	David W. Hamilton
2000	Bernard Robaire
2001	Gail S. Prins
2002	Terry T. Turner
2003	Arnold M. Belker
2004	J. Lisa Tenover
2005	Barry Hinton
2006	Barry Zirkin

young andrologist award


John Amory, MD

The Young Andrologist Award recognizes the contributions to the field of Andrology by a member of the American Society of Andrology under 45 years of age. Dr. John Amory of the University of Washington is this year's recipient. A native of the Pacific Northwest, Dr. Amory obtained his undergraduate degree *magna cum laude* in biology from Harvard University in 1989 and received his MD degree at the University of California, San Francisco, in 1994. He then completed his residency in internal medicine at the University of California, San Francisco before coming to the University of Washington in 1997. He is currently an associate professor of medicine in the Division of General Internal Medicine at the University of Washington, and also recently obtained a Masters degree in Public Health from the University of Washington. He presently works as an attending physician both on the inpatient medicine service as well as in the outpatient setting in the General Internal Medicine Clinic, and balances these responsibilities with an extensive involvement in clinical/translational research and significant teaching responsibilities.

Dr. Amory's research interests focus on the development of novel forms of oral androgen therapy and male hormonal contraceptives. Dr. Amory has demonstrated the efficacy of oral androgen therapy, and shown that hormones administered by this method are bioavailable and can restore normal blood levels of androgen. This methodology has refuted long-held beliefs regarding the suitability of oral androgen administration, and has applicability both for androgen replacement therapy and various contraceptive methodologies. Dr. Amory has been well funded by NIH as well as the pharmaceutical industry, and he also is a co-investigator in a NIH Contraceptive Center Grant for research in the area of male contraceptive development.

Dr. Amory's clinical and research accomplishments have been widely recognized by both the medical and scientific communities, as well as the popular press. He received the Paul Beeson Award for excellence in Housestaff Instruction from the Department of Medicine at the University of Washington Medical School in 2003. In 2004, he was named a Helen and Phillip Fialkow Scholar in the Department of Medicine at the University of Washington Medical School, and received the Endocrine Society International Award for Excellence in Published Clinical Research in 2005. He was also named as one of "Seattle's Best Doctors" by Seattle Magazine in 2006.

Dr. Amory has effectively combined responsibilities in clinical medicine and teaching with cutting edge research. He has excelled in all of these areas during his brief career, despite the extensive and unique demands of each. The American Society of Andrology is built on the twin pillars of clinical andrology and basic science research in male reproductive biology, and ASA is proud to recognize as this year's recipient of the Young Andrologist Award someone who excels in both of these areas.

Young Andrologist Award Recipients

1982	L.J.D. Zaneveld
1983	William B. Neaves
1984	Lonnie D. Russell
1985	Bruce D. Schanbacher
1986	Stephen J. Winters
1987	Ilpo T. Huhtaniemi
1988	Larry Johnson
1989	Barry T. Hinton
1990	Luis Rodriguez/Rigau
1991	Patricia M. Saling
1992	Gary R. Klinefelter
1993	Robert Chapin
1994	Wayne J.G. Hellstrom
1995	Christopher DeJonge
1996	Paul S. Cooke
1997	Gail A. Cornwall
1998	William R. Kelce
1999	Stuart E. Ravnik
2000	Matthew P. Hardy
2001	Jacquetta Trasler
2002	Christopher L.R. Barratt
2003	Joanna E. Ellington
2004	Kate Loveland
2005	Janice Bailey
2006	Janice P. Evans

The Young Andrologist Award is sponsored in part by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.

outstanding trainee investigator award

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

The recipient of the 2007 New Investigator Award will be announced during the Annual Business Meeting on Monday, April 23, 2007 at 5:30 p.m.

New Investigator Award Recipients

1983	Thomas T. Tarter
1984	Peter S. Albertson
1985	Randall S. Zane
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
1997	Loren D. Walensky
1998	Dolores D. Mruk
1999	Jacques J. Tremblay
2000	Jeffrey J. Lysiak
2001	Alexander T.H. Wu
2002	Ebtesam Attaya
2003	Mustafa Faruk Usta

Outstanding Trainee Investigator Award Recipients

2004	Darius Paduch
2005	Tara Barton
2006	Liwei Huang

thanks to donors, supporters and exhibitors

The American Society of Andrology gratefully acknowledges these contributors to the various ASA Endowment or Asset Funds*:

Gold Level
(Multiple or single contribution(s) greater than or equal to $10,000)
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(Multiple or single contribution(s) greater than or equal to $5,000)
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West Michigan Reproductive Institute
Women in Andrology

Sustaining
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Richard D. Amelar, MD
Rudi Ansbacher, MD
Arnold M. Belker, MD
Glenn R. Cunningham, MD
E. Mitch Eddy, PhD
Erwin Goldberg, PhD
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Christina Wang, MD

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Anyone interested in contributing to ASA Endowment or Asset Funds may contact the ASA office at **(847) 619-4909**.
** Contributions include waived payments of honoraria and awards donated to the Endowment Funds.*

2007 Exhibitors

The American Society of Andrology would like to acknowledge the following companies that will be exhibiting at the 32nd Annual Meeting.

(Alphabetical as of 1/5/07)

Auxilium Pharmaceuticals, Inc
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Thank you to our 2007 Industry Partners

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Texas Institute for Reproductive Medicine
SCSA

course objectives & CME credit information

andrology lab workshop

SPERM MORPHOLOGY: A HANDS-ON WORKSHOP

The Workshop will teach the two most popular morphology schemes used by fertility specialists today: the WHO 3rd Edition, based on a traditional classification of normal sperm morphology; and Strict Criteria, as described by lab scientists in Tygerberg and in the WHO 4th Edition where normal is defined according to very stringent criteria.

Overview

The Workshop will begin with an overview of sperm morphology classification including its history, relationship to fecundity, the rationale for different morphology schemes, and the downward trend of percent normal. Next, experts who use the WHO 3rd classification and the Strict (Tygerberg, WHO4th) morphology classification system will teach these methods. Sperm images from stained semen smears will be projected onto the classroom screen for analysis and the faculty will classify each sperm, while discussing classification rationale for each image. After the presentation, each participant will receive photos sperm images of virtual smears to perform a morphology assessment with the faculty present to help. Participants may keep the images for future reference and practice.

QUALITY CONTROL: A HANDS-ON WORKSHOP

Quality Control will be taught by Dr. Stephen Simon and Steven Schrader. Dr. Stephen Simon, a statistician, has been teaching quality assurance workshops for over 10 years. He is the author of a new book, entitled "Statistical Evidence in Medical Trials". Dr. Steven Schrader leads a research team conducting reproductive health assessments for the National Institute for Occupational Safety and Health. He is part of the team that is writing the quality control chapter of the forthcoming new edition of the WHO manual on semen analysis.

Overview

The afternoon workshop will then move into a presentation on quality control. Topics include establishing management support and measuring your process as well as implementing the management process in your laboratory. You will learn to use Pareto Charts, Cause and effect diagrams, flow diagrams and control charts for the various QC processes in the laboratory. Improve the quality in your laboratory by reducing variation, identify and remove special and common causes. Learn to use Analysis of Means (ANOM) to compare the results of the andrologists in your laboraratory. The workshop will conclude with a summary and moderated discussion. Ample time for discussion of all topics has been allocated.

Learning Objectives

- Recognize the history of sperm morphology and it's clinical significance
- Name and perform sperm classification assessments using WHO 3rd and Strict (Tygerberg, WHO4th) criteria
- Distinguish the differences between the two morphology classification systems.
- Interpret Pareto charts, Cause and Effect diagrams, flow diagrams and control charts.
- Integrate measures to identify common and special causes of variation and reduce them.
- Prepare Analysis of Means (ANOM) to compare among a peer group or analyze data from an experiment with multiple levels.

asa special symposium

Needs:

- Male hypogonadism occurs in between 2 to 4 million men in the United States, many of these patients are not diagnosed and never receive treatment. Physicians and providers need to accurately identify patients with male hypogonadism.
- Metabolic syndrome is one of the most common disorders in the adult populations and the cause and effect relationship is not recognized.
- Many new current and future therapies for androgen substitution are or will be available. Knowledge of these options will help in the management of these patients with hypogonadism.

Learning Objectives:

- Identify the symptoms and use appropriate tests to diagnose male hypogonadism.
- Recognize the relationships between components of the metabolic syndrome with androgen deficiency.
- Explain the different types of androgen replacement therapy.
- Illustrate the use of PDE 5 inhibitors with androgens in men with sexual dysfunction.

annual meeting

"On The Cutting Edge of Basic, Translational and Clinical Andrology: Implications for the Future of Male Reproductive Health"

Needs

Recent research has enabled advances in understanding, evaluation and treatment of male reproductive disorders and conditions, including prostate cancer, infertility, premature ejaculation, priapsim and Peyronnie disease. This program will provide andrology professionals, including male reproductive specialists, endocrinologists, urologists, gynecologists and obstetricians, biochemists and molecular scientists and cell biologists, with the most cutting-edge basic, translational and clinical research impacting current reproductive disorders and the future of male reproduction.

The presentation and discussion will allow andrology professionals to recognize and integrate information on technological and medical advancements of bioethics and genomics and varying aspects of male reproductive health into their practices. This program will also help andrologists identify innovations and developments in male fertility, such as spermatogenic stem cell therapy, influences on sperm function and contributors to prostate cancer growth.

Following this program, participants should be able to:

- Recognize the history of sperm morphology and it's clinical significance
- Recognize the implications of epigenetic regulation of development on stem cell therapy and cloning.
- Formulate an appreciation of the potential uses of proteomic and genomic signatures for prostate cancer screening, diagnosis and treatment.
- Describe recent knowledge regarding the role of acid-base transporters, ion channels and odorant receptors on epididymal and sperm function.
- Describe the development and potential uses of selective androgen receptor modulators.
- Formulate an appreciation of the technology, medicine and bioethics of preimplantation genetics.
- Explain the clinical implications of germ cell differentiation for male infertility.
- Describe recent advances in the pathophysiology and treatment of priapism, Peyronnie disease and premature ejaculation.
- Describe the role of protein kinase A in spermatogenesis and sperm function.
- Recognize the implications of DNA methylation on genomic imprinting, development and male infertility.
- Formulate an appreciation of new approaches in environmental epidemiology studies of male reproductive health.
- Recognize the hormonal changes that occur in men with aging and their clinical implications.
- Describe the role of testicular macrophages in testis function.
- Explain the role of apoptosis, the androgen receptor and vitamin D in regulation of prostate cancer growth.
- Formulate an appreciation of the clinical relevance of androgen receptor polymorphisms.
- Describe the role of extra-testicular Sertoli cells in transplantation.
- Describe the role of steroidogenic enzymes in germ cells on sperm function and fertility.
- Recognize the potential therapeutic uses of spermatogenic stem cells.

CME credit information

Accreditation
For accreditation updates, please check the website at www.andrologysociety.com.

Special Assistance
We encourage participation by all individuals. If you have a disability, advance notification of any special needs will help us better serve you. Call 847-619-4909 if you require special assistance to fully participate in the meeting.

mark your calendars!

ASA 33rd Annual Conference
April 12 - 15, 2008
Hyatt Regency Albuquerque
Albuquerque, NM

Andrology Lab Workshop:
April 12, 2008

schedule of events

XIX NORTH AMERICAN TESTIS WORKSHOP

Chromosome Structure and Gene Expression
Program Chair: Michael D. Griswold, PhD
Co-Chair: E. Mitch Eddy, PhD
April 18 – 21, 2007
**Location: Regency Ballroom 4-7 (unless otherwise noted)*

Registration fee includes entry into the workshop, a syllabus, continental breakfasts and refreshment breaks.

WEDNESDAY, APRIL 18, 2006

6:00 p.m. – 8:30 p.m.	**Registration** *Location: Regency Foyer*
7:00 p.m. – 7:15 p.m.	**Welcome Address** *Michael D. Griswold, PhD* *Washington State University*
7:15 p.m. – 8:30 p.m.	**Keynote Address: What We Have Learned From Sequencing Sex Chromosomes?** *David Page, PhD* *Whitehead Institute*

THURSDAY, APRIL 19, 2007

7:00 a.m. – 6:00 p.m.	**Registration** *Location: Regency Foyer*
7:30 a.m. – 8:30 a.m.	**Continental Breakfast** *Location: Regency Foyer*

SESSION I: SEX CHROMOSOMES, GENOMICS, EXPRESSION PROFILING
Chair: Mary Ann Handel, PhD

8:30 a.m. – 9:30 a.m.	**Benchmark Lecture: What Do Testis and Tumors Have In Common?** *Michael Primig* *University of Basal*
9:30 a.m. – 10:15 a.m.	**Gene Expression During Leydig Cell Development** *Matthew P. Hardy, PhD* *Population Council*
10:15 a.m. – 10:45 a.m.	**Break**
10:45 a.m. – 11:30 a.m.	**Tex Genes and Germ Cell Bridges** *Martin M. Matzuk, MD, PhD* *Baylor College of Medicine*
11:30 a.m. – 12:15 p.m.	**Transcriptional Profiling of the States of the Cycle of the Rat Seminiferous Epithelium and Purified Testicular Cells** *Daniel S. Johnston, PhD* *Wyeth Research*
12:15 p.m. – 1:30 p.m.	**Break**

SESSION II: STEM CELLS AND SPERMATOGONIA
Chair: Marvin L. Meistrich, PhD

1:30 p.m. – 2:15 p.m.	**In Vivo Actions of the Spermatogenic Stem Cells** *Soshei Yoshida* *Kyoto University*
2:15 p.m. – 3:00 p.m.	**Spermatogonial Stem Cell Renewal in Drosophila Testis** *Erika Matunis* *Johns Hopkins University*
3:00 p.m. – 3:30 p.m.	**Break**
3:30 p.m. – 4:15 p.m.	**Culture and Genetic Modification of Mouse Male Germline Stem (GS) Cells** *Takashi Shinohara, PhD* *Kyoto University*
4:15 p.m. – 5:00 p.m.	**Brief Presentations from Submitted Abstracts**
5:00 p.m.	**Poster Session I** *Location: Regency Ballroom 1-3*

FRIDAY, APRIL 20, 2007

7:00 a.m. – 5:00 p.m.	**Registration** *Location: Regency Foyer*
7:30 a.m. – 8:30 a.m.	**Continental Breakfast** *Location: Regency Foyer*

SESSION III: REGULATION OF GENE EXPRESSION
Chair: Leslie Lynn Heckert, PhD

8:30 a.m. – 9:30 a.m.	**Benchmark Lecture: Androgen Regulated Homeobox Genes** *Miles F. Wilkinson, PhD* *U. T. M. D. Anderson Cancer Center*
9:30 a.m. – 10:15 a.m.	**TAFS in Drosophila** *Margaret Fuller* *Stanford University*
10:15 a.m. – 10:45 a.m.	**Break**
10:45 a.m. – 11:30 a.m.	**Non-Coding RNAs of the Mammalian Testis** *Norman B. Hecht, PhD* *University of Pennsylvania*
11:30 a.m. – 12:15 p.m.	**Promoters, Insulators, and Regulation of Testis-Specific Transcription** *Prabhakara Reddi, PhD* *University of Virginia*
12:15 p.m. – 1:30 p.m.	**Break**

SESSION IV: SOMATIC CELLS OF THE TESTIS
Chair: Patricia A. Martin-DeLeon, PhD

1:30 p.m. – 2:15 p.m. **Sox 8 and Sertoli Cell Function**
Moira K. O'Bryan, BSc, PhD
Monash University

2:15 p.m. – 3:00 p.m. **Retinoid Action in the Testis**
Kwan-Hee Kim, PhD
Washington State University

3:00 p.m. – 3:30 p.m. **Break**

3:30 p.m. – 4:15 p.m. **Androgen Action in the Testis**
Robert Braun, PhD
University of Washington

4:15 p.m. – 5:00 p.m. **Brief Presentations from Submitted Abstracts**

5:00 p.m. **Poster Session II**
Location: Regency Ballroom1-3

6:00 p.m. **Reception for Testis Workshop Registrants**
Location: City Center

SATURDAY, APRIL 21, 2007

7:00 a.m. – 7:30 p.m. **Registration**
Location: Regency Foyer

7:30 a.m. – 8:30 a.m. **Continental Breakfast**
Location: Regency Foyer

SESSION V: CLINICAL CORRELATES
Chair: E. Mitch Eddy, PhD

8:30 a.m. – 9:30 a.m. **Benchmark Lecture: Developmental Model for the Pathogenesis of Testicular Germ Cell Cancer**
Ewa Rajpert-De Meyts, MD, PhD
Rigshosppitalet, Copenhagen

9:30 a.m. – 10:15 a.m. **The Role of Dead-End in Germ Cell Tumor Development**
Angabin Matin
U. T. M. D. Anderson Cancer Center

10:30 a.m. – 11:30 a.m. **Chromosomes and Expression in Testicular Germ Cell Tumors**
Leendert Looijenga, PhD
Erasmus Medical Center,
The Netherlands

2007 ANDROLOGY LAB WORKSHOP

Program Chair: Aniela Bollendorf, MT
Saturday, April 21, 2007
**Location: Esplanade (unless otherwise noted)*

7:00 a.m. – 5:00 p.m. **Registration**

SPERM MORPHOLOGY - A HANDS-ON WORKSHOP

Registration fee includes a Syllabus, Continental Breakfast, Refreshment Break and Lab Science Forum Luncheon

AAB Peer CEU Pre-approval Pending
CME approval Pending

*Locations subjects to change

8:00 a.m. – 8:30 a.m. **Continental Breakfast**

8:30 a.m. – 9:15 a.m. **Overview of Sperm Morphology Classification Systems**
Susan Rothmann, PhD, HCLD
Fertility Solutions, Inc.

9:15 a.m. – 9:35 a.m. **WHO 3rd Sperm Morphology Classification Method Interactive Instruction**
Aniela Bollendorf, MT
Cooper Institute for Reproductive Hormonal Disorders

9:35 a.m. – 10:00 a.m. **WHO 4th Sperm Morphology Classification Method (Strict Criteria) Interactive Instruction**
David Karabinus, PhD, HCLD
Genetics and IVF Institute

10:00 a.m. – 10:15 a.m. **Refreshment Break**

10:15 a.m. – 11:30 a.m. **Sperm Morphology - Self Paced Using Virtual Smears**
Angela Reese, BS
Fertility Solutions, Inc.
Aniela Bollendorf, MT
Cooper Institute for Reproductive Hormonal Disorders
David Karabinus, PhD, HCLD
Genetics and IVF Institute

11:45 p.m. – 1:15 p.m. **Laboratory Science Forum Luncheon**
Sperm Motility Enhancers, Do They Really Improve Outcomes?
William E. Roudebush, PhD, HCLD (ABB)
Beckman Coulter, Inc.

QUALITY CONTROL - A HANDS-ON WORKSHOP

Registration fee includes a Syllabus, Refreshment Break and Lab Science Forum Luncheon
Steve Simon, PhD - Children's Mercy Hospital
Steven Schrader, PhD - NIOSH, CDC

AAB Peer CEU Pre-approval Pending
CME approval Pending

11:45 p.m. – 1:15 p.m. **Laboratory Science Forum Luncheon**
(Lunch included)
Sperm Motility Enhancers, Do They Really Improve Outcomes?
William E. Roudebush, PhD, HCLD (ABB)
Beckman Coulter, Inc.

1:30 p.m. – 2:15 p.m. **Quality Management Process**
-Establish management support
-Measure your process
-Pareto Charts, Cause and effect diagrams, flow diagrams, control chart
-Experiment

2:15 p.m. – 3:00 p.m. **Quality Improvement**
-Reduce variation
-Identify and remove special causes
-Identify and remove common causes

3:00 p.m. – 3:15 p.m. **Refreshment Break**

3:15 p.m. – 4:30 p.m. **Quality Control – Consistent Performance**
- Analysis of Means (ANOM)
- Performance is compared among a peer group
- Analyze data from an experiment with multiple levels

schedule of events

ASA SPECIAL SYMPOSIUM

Androgen Deficiency in Men - Challenges and Solutions
April 21, 2007
Co-Chairs: Ridwan Shabsigh, MD
Ronald Swerdloff, MD
**Location: Regency Ballroom 4-7 (unless otherwise noted)*
Registration is complimentary (Limited Seating Available)
CME Approval Pending

SATURDAY, APRIL 21, 2007

1:00 p.m. – 1:30 p.m. — **Registration**
Location: Registration Counter, Ballroom Level

1:00 p.m. – 1:30 p.m. — **Box Lunch**
Location: Regency Ballroom 4-7

1:30 p.m. – 2:00 p.m. — **Challenges in the Diagnosis of Male Hypogonadism** (Problems with diagnosis based on symptoms and signs: problems with testosterone assays; how can we address these issues?)
-Glenn Cunningham, MD, Professor of Medicine, Baylor College of Medicine

2:00 p.m. – 2:30 p.m. — **Androgen Deficiency, Metabolic Syndrome and Obesity** (How does androgen deficiency affect metabolism? Relationship between low androgen levels and insulin resistance, obesity and metabolic syndrome; How common is this problem? Are there ways to prevent this?)
-Frances Hayes, MD, Assistant Professor of Medicine, Harvard Medical School, Massachusetts General Hospital

2:30 p.m. – 3:00 p.m. — **Androgen Therapy: Current Practice and Future Perspectives** (What are the preparations available and those in development; what are the advantages and problems with each preparation? What is a SARM and how will SARMs be used clinically? Other new modalities)
Ronald Swerdloff, MD, Professor of Medicine,
David Geffen School of Medicine at UCLA, Harbor-UCLA Medical Center.

3:00 p.m. – 3:30 p.m. — **Testosterone with PDE5 Inhibitors for ED** (Should Testosterone be used with PDE5 inhibitors for erectile dysfunction? How does the combination work? Are there future treatment?)
Ridwan Shabsigh, MD, Associate Professor of Urology,
New York-Presbyterian Hospital, Columbia University

3:30 p.m. – 4:00 p.m. — **Panel Discussion with Audience Participation**

ASA 32ND ANNUAL MEETING

"On The Cutting Edge of Basic, Translational and Clinical Andrology: Implications for the Future of Male Reproductive Health"
Program Chair: Alvin M. Matsumoto, MD
April 21 – 24, 2007
**Location: Regency Ballroom 4-7 (unless otherwise noted)*
Registration fee includes entry into the lectures, one ticket to the Welcome Reception, a syllabus, and refreshment break.

FRIDAY, APRIL 20, 2007

7:00 a.m. – 5:00 p.m. — **Registration**
Location: Registration Counter, Ballroom Level

4:00 p.m. – 11:00 p.m. — **Executive Council Meeting**
Location: Buccaneer A-B

SATURDAY, APRIL 21, 2007

7:00 a.m. – 7:30 p.m. — **Registration**
Location: Registration Counter, Ballroom Level

4:00 p.m. – 7:00 p.m. — **Exhibit Hall Open**
Location: Regency Ballroom 1-3

6:00 p.m. – 6:10 p.m. — **Welcome and Opening Remarks**
President: Christina Wang, MD
Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute
Local Arrangements Chair:
Don F. Cameron, PhD
University of South Florida
Program Chair: Alvin M. Matsumoto, MD
University of Washington

6:10 p.m. – 6:30 p.m. — **Distinguished Andrologist Award**

6:30 p.m. – 7:30 p.m. — **ASA KEYNOTE LECTURE**
Epigenetic Regulation of Mammalian Development: Implications for Stem Cell Therapy and Cloning
Rudolf Jaenisch, MD
Whitehead Institute, Massachusetts Institute of Technology
(Introduced by Christina Wang, MD)

7:30 p.m. – 9:30 p.m. — **Welcome Reception**
Location: City Center

SUNDAY, APRIL 22, 2007

6:30 a.m. – 8:00 a.m. — **Past President's Breakfast**
Location: Buccaneer A

7:00 a.m. – 4:00 p.m. — **Exhibit Hall Open**
Location: Regency Ballroom 1-3

7:00 a.m. – 6:00 p.m. — **Registration**
Location: Registration Counter, Ballroom Level

*Locations subjects to change

schedule of events

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
Use of Proteomic and Genomic Signatures for Prostate Cancer Screening, Diagnosis and Treatment
Arul M. Chinnaiyan, MD, PhD
University of Michigan Medical Center
(Introduced by Dana Ohl, MD)

9:00 a.m. – 9:15 a.m **Distinguished Service Award**

9:15 a.m. – 10:45 a.m. **SYMPOSIUM I – UPDATE ON EPIDIDYMAL AND SPERM FUNCTION: TRANSPORTERS, CHANNELS AND SENSORY RECEPTORS**
Co-chairs: E. Mitch Eddy, PhD and Kim Chau, BS

Acid-base Transporters in the Male Reproductive Tract: Potential Consequences of Malfunction
Sylvie Breton, PhD
Massachusetts General Hospital

Ion Channels and Sperm Function: Potential Clinical Implications
Timothy Quill, PhD
University of Texas-Southwestern

Odorant Receptor and Sperm Function: Do Sperm Have a Sense of Smell?
Marc Spehr, PhD
Ruhr-Universitaet Bochum

10:45 a.m. – 12:30 p.m. **Poster Session I**
Location: Regency Ballroom 1-3

12:30 p.m. – 2:00 p.m. **Lunch (on your own)**

12:30 p.m. – 2:00 p.m. **Women in Andrology Luncheon and Discussion**
(not included in registration fee; tickets required)
Location: Buccaneer B

2:00 p.m. – 3:30 p.m. **Concurrent Oral Sessions I and II**

Spermatogenesis, Infertility, Contraception and Sexual Function
Moderators: John K. Amory, MD and Michaela Luconi, PhD
Location: Regency 4-7

2:00 p.m. – Abstract 1
MRNA PROFILES IN SPERM OF PATIENTS WITH PROTAMINE DEREGULATION INDICATE A MORE GENERALIZED DEVELOPMENTAL PATHOLOGY
Benjamin R. Emery, BS, Andrology & IVF Laboratories, University of Utah School of Medicine, Stephen A. Krawetz, PhD, Department of OB-GYN, Wayne State University School of Medicine, Adrian E. Platts, BS, Department of OB-GYN, Wayne State University School of Medicine and Douglas T. Carrell, PhD, Andrology & IVF Laboratories, University of Utah School of Medicine (Presented By: Benjamin R. Emery, BS, Andrology & IVF Laboratories, University of Utah School of Medicine)

2:15 p.m. – Abstract 2
DISULFIDE BONDS OF SPERMATOGENIC CELL-SPECIFIC TYPE 1 HEXOKINASE (HK1S) AND SPERM MOTILITY
Noriko Nakamura, PhD, National Institute of Environmental Health Sciences, NIH and Edward M. Eddy, PhD, NIEHS/NIH (Presented By: Noriko Nakamura, PhD, National Institute of Environmental Health Sciences, NIH)

2:30 p.m. – Abstract 3
IMMUNE REGULATION IN HUMAN TESTIS
Maciej Kurpisz, MD, PhD, Institute of Human Genetics, Polish Academy of Sciences, Dorota Fiszer, PhD, Institute of Human Genetics, Poznan, Poland, Natalia Rozwadowska, PhD, Institute of Human Genetics, Poznan, Poland, Piotr Jedrzejczak, MD, Clinic of Infertility and Reproductive Endocrinology, Poznan Medical School and Wlodzimierz Kosicki, MD, Department of Surgery, State District Hospital, Poznan (Presented By: Maciej Kurpisz, MD, PhD, Institute of Human Genetics, Polish Academy of Sciences)

2:45 p.m. – Abstract 4
ETIOLOGY OF SEMINIFEROUS TUBULE BASEMENT MEMBRANE THICKENING (BMT) IN INFERTILE MEN WITH VARICOCELES
Susan Benoff, PhD, The Feinstein Institute for Medical Research, Ian Hurley, PhD, The Feinstein Institute for Medical Research and Joel Marmar, MD, Robert Wood Johnson School of Medicine (Presented By: Susan Benoff, PhD, The Feinstein Institute for Medical Research)

3:00 p.m. – Abstract 5
SIMILAR INTRATESTICULAR ANDROGEN CONCENTRATIONS IN MEN WITH SEVERE OLIGOSPERMIA VERSUS THOSE WITH POORLY SUPPRESSED SPERMATOGENESIS INDUCED BY A MALE HORMONAL CONTRACEPTIVE REGIMEN
Stephanie T. Page, MD, PhD, Department of Medicine, University of Washington School of Medicine, John K. Amory, MD, MPH, Department of Medicine, University of Washington, Seattle, WA, Thomas F. Kalhorn, PhD, University of Washington Mass Spectrometry Center, Bradley D. Anawalt, MD, Department of Medicine, University of Washington, and VAPSHCS, Seattle, WA, Alvin M. Matsumoto, MD, Department of Medicine, University of Washington, and VAPSHCS, Seattle, WA and William J. Bremner, MD, PhD, Department of Medicine, University of Washington, Seattle, WA (Presented By: Stephanie T. Page, MD, PhD, Department of Medicine, University of Washington School of Medicine)

3:15 p.m. – Abstract 6
UROSELECTIVE ALPHA-1 BLOCKER ALFUZOSIN RESTORED ERECTILE DYSFUNCTION AFTER PARTIAL BLADDER OUTLET OBSTRUCTION IN RATS
Serap Gur, PhD, Tulane, Suresh Sikka, PhD, Tulane University, Department of Urology and Wayne Hellstrom, MD, FACS, Tulane (Presented By: Suresh Sikka, PhD, Tulane University, Department of Urology)

Sperm Function, ART, Epididymis, and Fertilization
Moderators: Patricia A. Martin-DeLeon, PhD and Genevieve Griffiths, BA
Location: Garrison Suite

2:00 p.m. – Abstract 7
COMPLEMENTARY STUDY OF SPERM CHROMATIN QUALITY IN CANCER PATIENTS BY FLOW CYTOMETRY
Cristian O'Flaherty, DVM, PhD, McGill University, Peter Chan, MD, McGill University Health Centre, Farida Vaisheva, MD, McGill University, Barbara Hales, PhD, McGill University and Bernard Robaire, PhD, McGill University (Presented By: Cristian O'Flaherty, DVM, PhD, McGill University)

2:15 p.m. – Abstract 8
NYD-SP27, AN INTRINSIC DECAPACITATION FACTOR IN SPERM
Ye Bi, Laboratory of Reproductive medicine, Department of Histology and Embryology, Nanjing Medical University, Ying Lu, Master, Nanjing Medical University, Wenming Xu, Master, The Chinese University of Hong Kong, Hau Yan Wong, Master, The Chinese University of Hong Kong, Zuomin Zhou, Doctor, Nanjing Medical University, Hsiao Chang Chan, Doctor, The Chinese University of Hong Kong and Jia Hao Sha, Doctor, Nanjing Medical University (Presented By: Ye Bi, Laboratory of Reproductive medicine, Department of Histology and Embryology, Nanjing Medical University)

2:30 p.m. – Abstract 9
IMPROVED SPERM CHROMATIN DECONDENSATION RATES IN HAMSTER-OOCYTE ICSI AFTER ANNEXIN-V-MACS IN INFERTILITY PATIENTS
Sonja Grunewald, MD, EAA Center, University of Leipzig, Verona Blumenauer, Dipl-Biol, Clinic of Reproductive Medicine, Leipzig, Germany, Martin Reinhardt, cand med, EAA Center, University of Leipzig, Germany, Ashok Agarwal, Professor, Reproductive Research Center, Cleveland Clinic Foundation, Cleveland, USA, Tamer Said, MD, Toronto Institute of Reproductive Medicine, Toronto, Canada, Fayez Abu Hmeidan, MD, Clinic of Reproductive Medicine, Leipzig, Germany, Hans-Juergen Glander, Professor, EAA Center, University of Leipzig, Germany and Uwe Paasch, MD, PhD, EAA Center, University of Leipzig, Germany (Presented By: Sonja Grunewald, MD, EAA Center, University of Leipzig)

2:45 p.m. – Abstract 10
MULTIPLE STEPS IN THE FERTILIZATION CASCADE ARE IMPAIRED WHEN SPERM GLYCOLYSIS IS DISRUPTED
ZAOHUA HUANG, PhD, UNC at Chapel Hill, Kathy Mohr, BS, UNC at Chapel Hill and Deborah O'Brien, PhD, UNC at Chapel Hill (Presented By: ZAOHUA HUANG, PhD, UNC at Chapel Hill)

3:00 p.m. – Abstract 11
PROTEOMIC ANALYSIS OF EPIDIDYMAL CLEAR CELLS AND V-ATPASE-ASSOCIATED PROTEINS
Nicolas Da Silva, PhD, Massachusetts General Hospital - Harvard Medical School, Winnie Shum, PhD, Massachusetts General Hospital - Harvard Medical School, Vladimir Marshansky, PhD, Massachusetts General Hospital - Harvard Medical School, Dennis Brown, PhD, Massachusetts General Hospital - Harvard Medical School and Sylvie Breton, PhD, Massachusetts General Hospital - Harvard Medical School (Presented By: Nicolas Da Silva, PhD, Massachusetts General Hospital - Harvard Medical School)

3:15 p.m. – Abstract 12
EVIDENCE FOR THE INVOLVEMENT OF TESTICULAR PROTEIN CRISP2 IN MOUSE SPERM-EGG FUSION
Dolores Busso, PhD, Instituto de Biologìa y Medicina Experimental (IBYME-CONICET), Debora Cohen, PhD, Instituto de Biologia y Medicina Experimental, Nadia Goldweic, MSc, Instituto de Biologìa y Medicina Experimental (IBYME-CONICET), Julieta Maldera, MSc, Instituto de Biologìa y Medicina Experimental (IBYME-CONICET) and Patricia Cuasnicu, PhD, Instituto de Biologìa y Medicina Experimental (IBYME-CONICET) (Presented By: Debora Cohen, PhD, Instituto de Biologia y Medicina)

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Regency Foyer

4:00 p.m. – 4:45 p.m. **LECTURE I**
Development and Potential Uses of Selective Androgen Receptor Modulators (SARM's)
James T. Dalton, PhD
Ohio State University
(Introduced by Glenn R. Cunningham, MD)

4:45 p.m. – 5:30 p.m. **LECTURE II:**
Preimplantation Genetics: The Technology, The Medicine and The Bioethics
Masaru Okabe PhD
Osaka University
(Introduced by Janice L. Bailey, PhD)

6:00 p.m. – 8:00 p.m. **Trainee Forum and Mixer**
(All Trainee Travel Awards will be distributed and celebrated at this event)
Location: Buccaneer A

MONDAY, APRIL 23, 2007

7:00 a.m. – 12:30 p.m. **Exhibit Hall Open**
Location: Regency Ballroom 1-3

7:00 a.m. – 6:00 p.m. **Registration**
Location: Registration Counter, Ballroom Level

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Human Germ Cell Differentiation: Clinical Implications for Male Infertility
Renee A. Reijo Pera, PhD
University of California at San Francisco
(Introduced by Mary M. Lee, MD)

9:00 a.m. – 9:15 a.m. **Young Andrologist Award**

9:15 a.m. – 10:45 a.m. **SYMPOSIUM II – CLINICAL UROLOGY UPDATE: TOO LONG, TOO CURVED AND TOO QUICK**
Co-chairs: Wayne J.G. Hellstrom, MD and Peter Liu, MBBS, PhD

Basic Insights into the Pathophysiology of Priapism: Enough Already
Arthur L. Burnett, MD
Johns Hopkins University

Molecular and Cellular Basis of Peyronie Disease: Mr. Snappy of the Penis
Jacob Rajfer, MD
UCLA Medical Center

Advances in the Pathophysiology and Pharmacotherapy of Premature Ejaculation: Slow Down You're Moving Too Fast
Ridwan Shabsigh, MD
Columbia University

10:45 a.m. – 12:30 p.m. **Poster Session II**
Location: Regency 1-3

12:30 p.m. – 2:00 p.m. **Lunch (on your own)**

12:30 p.m. – 2:00 p.m. **MENTORING LUNCHEON SPONSORED BY THE DIVERSITY, TRAINEE AFFAIRS AND ETHICS COMMITTEES**
Ethics in Andrology
Stanley G. Korenman, MD
David Geffen School of Medicine at UCLA
Location: City Center
(not included in registration; tickets required)

schedule of events

12:30 p.m. – 2:00 p.m. **Editorial Board Luncheon**
Location: Harborview - 16th Floor

2:00 p.m. – 3:30 p.m. **SYMPOSIUM III – SPERMATOGENESIS, MALE INFERTILITY AND REPRODUCTIVE TOXICOLOGY**
Co-chairs: Kenneth P. Roberts, PhD and Sarika Saraswati, MS

Role of Protein Kinase A in Spermatogenesis and Sperm Function: of Mice and Men
G. Stanley McKnight, PhD
University of Washington

DNA Methylation: Implications for Genomic Imprinting, Development and Male Infertility
Jacquetta Trasler, MD, PhD
McGill University, Montreal, Canada

Novel Approaches for Conducting Environmental Epidemiology Studies on Male Reproductive Health
Sally Perreault Darney, PhD
US Environmental Protection Agency

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Regency Foyer

4:00 p.m. – 4:45 p.m. **LECTURE III**
(European Academy of Andrology Lecture)
Location: Regency Ballroom 1-3
The European Male Aging Study: What Happens as Men Age and What Are the Implications?
Frederick C.W. Wu, MD
University of Manchester, United Kingdom
(Introduced by William J. Bremner, MD, PhD)

4:45 p.m. – 5:30 p.m. **LECTURE IV**
Location: Regency Ballroom 1-3
Testicular Macrophages: Friends or Enemies Within
James C. Hutson, PhD
Texas Tech University
(Introduced by Kate Loveland, PhD)

5:30 p.m. **ASA Business Meeting**
Location: Regency Ballroom 1-3
Outstanding Trainee Investigator and Trainee Awards

7:30 p.m. – 10:30 p.m. **Annual Banquet**
(not included in registration fee; tickets required)
Location: Starship Cruise
(Boat sails at 8:00 p.m.)

TUESDAY, APRIL 24, 2007

7:00 a.m. – 8:00 a.m. **2008 Program Committee Meeting**
Location: Channelside 1

7:30 a.m. – 11:00 a.m. **Registration**
Location: Registration Counter, Ballroom Level

8:00 a.m. – 9:30 a.m. **SYMPOSIUM IV – REGULATION OF PROSTATE CANCER GROWTH**
Location: Regency Ballroom 1-3
Co-Chairs: Gail S. Prins, PhD and Stephanie Page, MD, PhD

Regulation of Apoptosis and Prostate Cancer Growth
Natasha Kyprianou, MD, PhD
University of Kentucky Medical Center

Androgen Receptor and Control of Prostate Cancer Growth
Norman Greenberg, PhD
Fred Hutchinson Cancer Research Center, Seattle, Washington

Vitamin D Regulation of Prostate Cancer Growth: Clinical Implications
Kerry L. Burnstein, PhD
University of Miami School of Medicine

9:30 a.m. – 9:45 a.m. **Break**
Location: Regency Foyer

9:45 a.m. – 10:45 a.m. **INTERNATIONAL LECTURE**
Location: Regency Ballroom 1-3
Clinical Relevance of Androgen Receptor Polymorphism
Eberhard Nieschlag, MD
University of Muenster, Germany
(Introduced by Patricia S. Cuasnicu, PhD)

10:45 a.m. – 12:15 p.m. **SYMPOSIUM V – INTRAMURAL AND EXTRAMURAL TESTIS CELL BIOLOGY**
Location: Regency Ballroom 1-3
Co-chairs: Don F. Cameron, PhD and Monica Schwarcz, MD

Extra-testicular Sertoli Cell: Immunobiology and Role Transplantaion
Jannette Dufour, PhD
Texas Tech University

Role of Steroidogenic Enzymes in Germ Cells on Sperm Function and Fertility
Vassilios Papadopoulos, PhD
Georgetown University

Spermatogonial Stem Cells in Primate Testes: Potential Therapeutic Uses
Stefan Schlatt, PhD
University of Pittsburgh School of Medicine

poster session 1

Sunday, April 22, 2007
10:45 a.m. – 12:30 p.m.
Location: Regency Ballroom 1-3

ANDROGENS/ENDOCRINOLOGY

Poster# 13 **INVOLVEMENT OF JNK AND P38 MAP KINASE SIGNALING IN CARDIOTOXIN- INDUCED MUSCLE INJURY: ROLE OF TESTOSTERONE**
Indrani Sinha-Hikim, PhD, Charles R. Drew University of Medicine and Science, Ruoqing Shen, MD, Charles R. Drew, Benjamine Tran, MS, Charles R. Drew and Melissa Braga, BS, Charles R. Drew (Presented By: Indrani Sinha-Hikim, PhD, Charles R. Drew, University of Medicine and Science)

Poster# 14 **CALCITRIOL (1ALPHA,25-DIHYDROXYVITAMIN D3) INHIBITS ANDROGEN GLUCURONIDATION IN PROSTATE CANCER LNCAP CELLS**
Jenny Kaeding, MSc, Molecular Endocrinology and Oncology, CHUL Research Center; Faculty of pharmacy, Laval University, Québec, Canada, Julie Bélanger, Molecular Endocrinology and Oncology, CHUL Research Center; Faculty of pharmacy, Laval University, Québec, Canada, Alain Bélanger, PhD, Molecular Endocrinology and Oncology, CHUL Research Center; Faculty of pharmacy, Laval University, Québec, Canada and Olivier Barbier, PhD, Molecular Endocrinology and Oncology, CHUL Research Center; Faculty of pharmacy, Laval University, Québec, Canada (Presented By: Jenny Kaeding, MSc, Molecular Endocrinology and Oncology, CHUL Research Center; Faculty of pharmacy, Laval University)

Poster# 15 **LEPTIN AND GHRELIN BALANCE IS REQUIRED FOR MURINE**
Shannon Whirledge, BS & BA, Baylor College of Medicine, Roy Smith, PhD, Baylor College of Medicine and Dolores Lamb, PhD, Baylor College of Medicine (Presented By: Shannon Whirledge, BS & BA, Baylor College of Medicine)

SPERMATOGENESIS/STEROIDOGENESIS/TESTIS BIOLOGY

Poster# 16 **EFFECT OF SUBCLINICAL AND OVERT HYPOTHYROIDISM ON TESTICULAR STEROIDOGENESIS**
Anand Kumar, MD, MAMS, All India Institute of Medical Sciences, Bidut Mohanty, PhD, All India Institute of Medical Sciences and Lata Rani, Msc, All India Institute of Medical Sciences (Presented By: Anand Kumar, MD, MAMS, All India Institute of Medical Sciences)

Poster# 17 **FUNCTIONAL STUDY OF HT31-8, A NOVEL HUMAN SPERMATOGENESIS-RELATED GENE**
Hui Zhu, PhD, Key lab of Reproductive Medicine, Nanjing Medical University, Zuomin Zhou, PhD, Key lab of Reproductive Medicine, Nanjing Medical University, Min Lin, Key lab of Reproductive Medicine, Nanjing Medical University, Jianmin Li, PhD, Deparment of Biology, Nanjing Medical University and Jaiohao Sha, PhD, Key lab of Reproductive Medicine, Nanjing Medical University (Presented By: Hui Zhu, PhD, Key lab of Reproductive Medicine, Nanjing Medical University)

Poster# 18 **INFLUENCE OF ANDROGENS ON TESTICULAR CONNEXINS IN BROOK TROUT**
Benjamin de Montgolfier, MSc, INRS-Institut Armand Frappier, Aliou Faye, MSc, ISMER-UQAR, Céline Audet, Professor, ISMER-UQAR and Daniel G. Cyr, Professor, INRS-Institut Armand Frappier (Presented By: Benjamin de Montgolfier, MSc, INRS-Institut Armand Frappier)

Poster# 19 **EVIDENCE THAT ANDROGEN SUPPRESSION INDUCED STIMULATION OF SPERMATOGONIAL DIFFERENTIATION IN JSD MICE ACTS BY ELEVATING THE SCROTAL TEMPERATURE**
Gunapala Shetty, PhD, University of Texas, M.D. Anderson Cancer Center, Connie Weng, MD, PhD, University of Texas, M.D. Anderson Cancer Center, Wei Zhou, PhD, University of Texas M.D. Anderson Cancer Center, Jun Ju, BS, University of Texas, M.D. Anderson Cancer Center and Marvin Meistrich, PhD, University of Texas, M.D. Anderson Cancer Center (Presented By: Gunapala Shetty, PhD, University of Texas M.D. Anderson Cancer Center)

Poster# 20 **ROLE OF CASPASE 2 IN APOPTOTIC SIGNALING OF PRIMATE MALE GERM CELLS**
Candace Johnson, BS, Yue Jia, MD, PHD, Amiya Sinha-Hikim, PHD, Ronald Swerdloff, MD, Yanhe Lue, MD, Xue Han, MD, Xue-Sen Zhang, MD, Zhao-Yuan Hu, MD, Yin-Chuan Li, MD, Yi-Xun Liu, MD and Christina Wang, MD (Presented By: Candace Johnson, BS)

INFERTILITY/ASSISTED REPRODUCTIVE TECHNOLOGY/MALE CONTRACEPTION

Poster# 21 **TESTICULAR HYPERTHERMIA BUT NOT HORMONE DEPRIVATION RESULTS IN STAGE-AND CELL-SPECIFIC ACTIVATION OF ERK AND INACTIVATION OF BCL-2**
Jesse Castellanos, BS, Yue Jia, MD, PhD, Jonathon Meyer, BS, Yanhe Lue, MD, Christina Wang, MD, Ronald Swerdloff, MD and Amiya Sinha Hikim, PhD (Presented By: Jesse Castellanos, BS)

Poster# 22 **ELEVATED SERUM INSL3 IS ASSOCIATED WITH FAILURE TO COMPLETELY SUPPRESS SPERMATOGENESIS IN MEN RECEIVING MALE HORMONAL CONTRACEPTION**
John Amory, MD, MPH, University of Washington, Stephanie Page, MD, PhD, University of Washington, Bradley Anawalt, MD, VA-Puget Sound, Alvin Matsumoto, MD, VA-Puget Sound and William Bremner, MD, PhD, University of Washington (Presented By: John Amory, MD, MPH, University of Washington)

poster session 1

Poster# 23 **ROLE OF JNK IN TESTICULAR CELLS AFTER HYPERTHERMIA AND/OR INSTRATESTICULAR TESTOSTERONE DEPRIVATION TREATMENTS IN ADULT CYNOMOLGUS MONKEYS**

Yue Jia, MD&PhD, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Amiya P. Sinha Hikim, PhD, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Ronald S. Swerdloff, MD, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Yanhe Lue, MD&PhD, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Candace Johnson, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Naomi Chiang, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Xiaodan Han, BS, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Yanira Vera, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Xueshen Zhang, PhD, Key Laboratory of Reproductive Biology, Chinese Academy of Science, Zhaoyuan Hu, PhD, Key Laboratory of Reproductive Biology, Chinese Academy of Science, Yinchuan Li, PhD, Key Laboratory of Reproductive Biology, Chinese Academy of Science, Yixun Liu, MD&PhD, Key Laboratory of Reproductive Biology, Chinese Academy of Science and Christina Wang, MD&PhD, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute (Presented By: Yue Jia, MD&PhD, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute)

Poster# 24 **PRE-TREATMENT OF SPERM WITH LOW HYPO-OSMOTIC SWELLING TESTS WITH CHYMOTRYPSIN PRIOR TO INTRAUTERINE INSEMINATION (IUI) AND AVOIDANCE OF UNPROTECTED INTERCOURSE RESULTS IN PREGNANCY RATES COMPARABLE TO IUI FOR OTHER MALE FACTOR PROBLEMS**

Gabrielle Citrino, UMDNJ, Robert Wood Johnson Med. School at Camden, Jerome Check, MD, PhD, UMDNJ, Robert Wood Johnson Med. School at Camden, Ann DiAntonio, BS, MT, UMDNJ, Robert Wood Johnson Med. School at Camden, Aniela Bollendorf, MT, UMDNJ, Robert Wood Johnson Med. School at Camden and Diane Katsoff, BS, MT, UMDNJ, Robert Wood Johnson Med. School at Camden (Presented By: Gabrielle Citrino, UMDNJ, Robert Wood Johnson Med. School at Camden)

Poster# 25 **ASSOCIATION OF SPERM DNA CYTOMETRY WITH ICSI OUTCOME**

Reda Mahfouz, MD, Cleveland clinic, Mona Elshafei, MD, PhD, Menofyia University, Amr Fathi, MD, PhD, Menofyia University, Rakesh Sharma, PhD, Cleveland Clinic, Medhat Amer, MD, PhD, Cairo Universit and Ashok Agarwal, PhD, Cleveland clinic (Presented By: Reda Mahfouz, MD, Cleveland Clinic)

Poster# 26 **EVALUATION OF MALE FACTOR AS A CAUSE OF SLOW CLEAVAGE BY EVALUATING INFERTILE DONOR/RECIPIENT PAIRS HAVING FRESH EMBRYO TRANSFER (ET)**

Brittney Katsoff, UMDNJ, Robert Wood Johnson Med. School at Camden, Jerome Check, MD, PhD, UMDNJ, Robert Wood Johnson Med. School at Camden, Carrie Wilson, BA, UMDNJ, Robert Wood Johnson Med. School at Camden, Aniela Bollendorf, MT, UMDNJ, Robert Wood Johnson Med. School at Camden and Diane Katsoff, BS, MT, UMDNJ, Robert Wood Johnson Med. School at Camden (Presented By: Brittney Katsoff, UMDNJ, Robert Wood Johnson Med. School at Camden)

Poster# 27 **IDIOPATHIC MALE INFERTILITY IS RELATED WITH GAMETOGENESIS GENES EXPRESSION: RESULTS BY A FUNCTIONAL ANALYSIS OF GENE ONTOLOGY TERMS.**

Nicolas Garrido, PhD, Instituto Universitario IVI, Jose Antonio Martinez-Conejero, PhD, Instituto Universitario IVI, Valencia, Spain, Juliana Jauregui, PhD, Instituto Universitario IVI, Valencia, Spain, Rakesh Sharma, PhD, Reproductive Research Center, Cleveland Clinic, Cleveland, OH, Jose Antonio Horcajadas, PhD, Instituto Universitario IVI, Valencia, Spain, Jose Remohi, MD, Instituto Universitario IVI, Valencia, Spain, Antonio Pellicer, MD, Instituto Universitario IVI, Valencia, Spain and Marcos Meseguer, PhD, Instituto Universitario IVI, Valencia, Spain (Presented By: Nicolas Garrido, PhD)

Poster# 28 **ANIMAL MODEL STUDY OF A NEW PATIENT LUBRICANT'S AFFECT ON IN VITRO FERTILIZATION & EMBRYO DEVELOPMENT**

Raymond Wright, PhD, Washington State University and Julie Schimmels, Washington State University (Presented By: Raymond Wright, PhD, Washington State University)

Poster# 29 **THE SIGNIFICANCE OF SPERM DNA OXIDATION IN EMBRYO DEVELOPMENT AND REPRODUCTIVE OUTCOME IN AN OOCYTE DONATION PROGRAM; A NEW MODEL TO STUDY MALE INFERTILITY PROGNOSTIC FACTOR.**

Marcos Meseguer, PhD, Instituto Universitario IVI, University of Valencia, Valencia, Spain, Jose A Martínez Conejero, PhD, Instituto Universitario IVI, University of Valencia, Valencia, Spain, Enrique O´Connor, PhD, Centro de Investigación Príncipe Felipe , Valencia, Spain, Ashok Agarwal, PhD, Reproductive Research Center, Cleveland Clinic, Cleveland, OH, Jose Remohí, MD, Instituto Universitario IVI, University of Valencia, Valencia, Spain, Antonio Pellicer, MD, Instituto Universitario IVI, University of Valencia, Valencia, Spain and Nicolás Garrido, PhD, Instituto Universitario IVI, University of Valencia, Valencia, Spain (Presented By: Marcos Meseguer, PhD, Instituto Universitario IVI, University of Valencia, Valencia, Spain)

Poster# 30 **PATERNAL DNA DAMAGE SUPPRESSES IN VITRO PROLIFERATION OF MOUSE INNER CELL MASS**

Satish Kumar Adiga, PhD, Division of Reproductive Medicine, Megumi Toyoshima, PhD, 2Kyoto University, Tsutomu Shimura, PhD, 2Kyoto University, Jun Takeda, MS, 2Kyoto University, Norio Uematsu, PhD, 2Kyoto University, Pratap Kumar, MD, Kasturba Medical College and Ohtsura Niwa, PhD, 2Kyoto University (Presented By: Satish Kumar Adiga, PhD, Division of Reproductive Medicine)

Poster# 31 **LOWER SPERM DENSITY IS ASSOCIATED WITH HIGHER CALORIE AND FAT INTAKE**

Lynn Wallock-Montelius, MS, PhD, Children's Hospital Oakland Research Institite (CHORI), Jodi Stookey, PhD, CHORI, Janet King, PhD, CHORI, UCB, Robert Jacob, PhD, USDA and Bruce Ames, PhD, CHORI, UCB (Presented By: Lynn Wallock-Montelius, MS, PhD, Children's Hospital Oakland Research Institite (CHORI)

Poster# 32 **CHARACTERIZATION OF THE PROTEOLYTIC ACTIVITY IN HUMAN SEMEN THAT REGULATES GALECTIN-3**
Sarika Saraswati, MS, University of Arkansas for Medical Sciences, John Platts, BS, Ashley S. Block, BS and Alan B. Diekman, PhD (Presented By: Sarika Saraswati, MS, University of Arkansas for Medical Sciences)

Poster# 33 **SIGNIFICANT DECREASE IN SPERM DEOXYRIBONUCLEIC ACID FRAGMENTATION AFTER VARICOCELECTOMY**
Donald Evenson, PHD HCLD, SCSA Diagnostics, Regina Wixon, PHD, SCSA Diagnostics, Kay Kasperson, BS, SCSA Diagnostics and Philip Werthman, MD FACS, Ctr for Male Reproductive Medicine (Presented By: Donald Evenson, PHD HCLD, SCSA Diagnostics)

Poster# 34 **CONCEIVING A CHILD AFTER VASECTOMY**
R.P.J. Schroeder, MSc, Dept. of Urology, University Medical Centre Utrecht, Utrecht, the Netherlands, M.L. Bots, MD, PhD, Julius Center for Health sciences and Primary Care, University Medical Centre Utrecht, Utrecht, the Netherlands, V.F. De Kemp, MSc, Dept. of Urology, University Medical Centre Utrecht, Utrecht, the Netherlands, P.M.M. Kastrop, PhD, Dept. of Reproductive Medicine, University Medical Centre Utrecht, Utrecht, the Netherlands and M.T.W.T. Lock, MSc, Dept. of Urology, Central Military Hospital and University Medical Centre Utrecht, Utrecht, the Netherlands (Presented By: R.P.J. Schroeder, MSc, Dept. of Urology, University Medical Centre Utrecht, Utrecht, the Netherlands)

SPERM FUNCTION/SEMEN ANALYSIS

Poster# 35 **CO-LOCALIZATION OF SINGLE-STRANDED DNA AND HISTONE H2B IN HUMAN SPERM NUCLEI: INSIGHT ON THE ETIOLOGY OF SPERM DNA DAMAGE**
Maria San Gabriel, PhD, McGill University, Xiaoyang Zhang, MD, McGill University, Jamie Libman, MD, McGill University and Armand Zini, MD, McGill University (Presented By: Armand Zini, MD, McGill University)

Poster# 36 **TEMPERATURE-DEPENDENT COLD SHOCK DAMAGE TO PORCINE SPERM CORRELATES WITH PROTEIN TYROSINE PHOSPHORYLATION***
Hannah Galantino-Homer, VMD, PhD, DACT, University of Pennsylvania School of Veterinary Medicine, Mark Modelski, BS, University of Pennsylvania School of Veterinary Medicine and Ina Dobrinski, Drmedvet, PhD, DACT, University of Pennsylvania School of Veterinary Medicine (Presented By: Hannah Galantino-Homer, VMD, PhD, DACT, University of Pennsylvania School of Veterinary Medicine)

Poster# 37 **COMPUTER ASSISTED SPERM HEAD MORPHOLOGY ASSESSMENT AND ITS CORRELATION WITH SPERM DNA DAMAGE**
Hussein Abdelrazik, MD, Cleveland Clinic, Reda Mahfouz, MD, Cleveland Clinic, Amr Abdelkader, MD, Cleveland Clinic, Rakesh Sharma, PhD, Cleveland Clinic, Sajal Gupta, MD, Cleveland Clinic and AshokAgarwal, PhD HCLD, Cleveland Clinic (Presented By: Hussein Abdelrazik, MD, Cleveland Clinic)

Poster# 38 **SIGNALING & SIGNALING RECEPTOR TRANSCRIPTS: DISRUPTION IN SEVERE HUMAN TERATOZOOSPERMIA**
Adrian Platts, BSc, Wayne State University and Stephen Krawetz, BSc PhD, Wayne State University, Medical School (Presented By: Adrian Platts, BSc, Wayne State University)

Poster# 39 **NORMAL SPERM MOTILITY REQUIRES THE PRESENCE OF JUNCTIONAL ADHESION MOLECULE-A (JAM-A)**
Minghai Shao, PhD, University of Delaware, Ulhas Naik, PhD, U. Delaware and Patricia Martin-DeLeon, PhD, U. Delaware (Presented By: Minghai Shao, PhD, University of Delaware)

Poster# 40 **A RECOMBINANT SEMINAL PROTEIN COMBATS INHIBITORY EFFECTS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS)**
Amy Cropp, BS, TMI Laboratories International Inc., Tod McCauley, PhD, TMI Laboratories International Inc., Sheldon Marks, MD, TMI Laboratories International Inc. and Roy Ax, PhD, TMI Laboratories International Inc. (Presented By: Amy Cropp, BS, TMI Laboratories International Inc.)

Poster# 41 **IDENTIFICATION OF SRC TYROSINE KINASE IN HUMAN SPERMATOZOA AND ITS ROLE DURING CAPACITATION**
Gabriele Varano, graduating student, Dept. Clinical Physiopathology-University of Florence, Adriana Lombardi, PhD student, Dept. Clinical Physiopathology-University of Florence, Gianni Forti, MD, Dept. Clinical Physiopathology-University of Florence, Elisabetta Baldi, PhD, Dept. Clinical Physiopathology-University of Florence and Michaela Luconi, PhD, Dept. Clinical Physiopathology-University of Florence (Presented By: Michaela Luconi, PhD, Dept. Clinical Dept. Clinical Physiopathology-University of Florence)

Poster# 42 **THE HEALTHY MEN STUDY: AN EVALUATION OF EXPOSURE TO WATER DISINFECTION BY-PRODUCTS AND SPERM QUALITY**
Tom Luben, PhD, MSPH, University of North Carolina at Chapel Hill, Andrew Olshan, PhD, University of North Carolina at Chapel Hill, Amy Herring, ScD, University of North Carolina at Chapel Hill, Susan Jeffay, BS, U.S. Environmental Protection Agency, Research Triangle Park, NC, Lillian Strader, BS, US Environmental ProtectionAgency, Research Triangle Park, NC, Ronna Chan, MPH, University of North Carolina at Chapel Hill, David Savitz, PhD, Mount Sinai School of Medicine and Sally Perreault, PhD, US Environmental Protection Agency, Research Triangle Park, NC (Presented By: Tom Luben, PhD, MSPH, University of North Carolina at Chapel Hill)

Poster# 43 **ANALYSIS OF THE ROLE OF INPP5B IN SPERM FUNCTION AND MALE INFERTILITY**
Matthew Marcello, BS, Johns Hopkins University/Division of Reproductive Biology and Janice Evans, PhD, Johns Hopkins University (Presented By: Matthew Marcello, BS, Johns Hopkins University/Division of Reproductive Biology)

poster session 1

Poster# 44 **OUABAIN STIMULATES PROTEIN PHOSPHORYLATION IN RAT SPERMATOZOA VIA THE NA,K-ATPASE.**
Gladis Sanchez, MD, University of Kansas Medical Center, Anh-Nguyet Nguyen, BS, University of Kansas Medical Center and Gustavo Blanco, MD, PhD, University of Kansas Medical Center (Presented By: Gustavo Blanco, MD, PhD, University of Kansas Medical Center)

Poster# 45 **BETA-CYCLODEXTRIN PLUS CHOLESTEROL REVERSIBLY INHIBITS PORCINE SPERM CAPACITATION***
Hannah Galantino-Homer, VMD, PhD, DACT, University of Pennsylvania School of Veterinary Medicine, Mark Modelski, BS, University of Pennsylvania School of Veterinary Medicine and Ina Dobrinski, Drmedvet, PhD, DACT, University of Pennsylvania School of Veterinary Medicine (Presented By: Hannah Galantino-Homer, VMD, PhD, DACT, University of Pennsylvania School of Veterinary Medicine)

FERTILIZATION/GERM CELL DEVELOPMENT/REPRODUCTIVE DEVELOPMENT

Poster# 46 **MULTIPLE STEPS IN THE FERTILIZATION CASCADE ARE IMPAIRED WHEN SPERM GLYCOLYSIS IS DISRUPTED**
ZAOHUA HUANG, PhD, UNC at Chapel Hill, Kathy Mohr, BS, UNC at Chapel Hill and Deborah O'Brien, PhD, UNC at Chapel Hill (Presented By: ZAOHUA HUANG, PhD, UNC at Chapel Hill)

Poster# 47 **EPIDIDYMOSOMES AND UTEROSOMES: THEIR ROLE IN SPERM UPTAKE OF GPI-LINKED PROTEINS**
Genevieve Griffiths, BA Biological Science, University of Delaware and Patricia Martin-DeLeon, PhD (Presented By: Genevieve Griffiths, BA Biological Science, University of Delaware)

Poster# 48 **STABILITY OF MEMBRANE ASSOCIATED ALPHA-L-FUCOSIDASE IN HUMAN SPERM CELLS**
Jennifer Venditti, BS, Lehigh University and Barry Bean, PhD, Lehigh University
(Presented By: Jennifer Venditti, BS, Lehigh University)

Poster# 49 **LOCALISATION OF P80, THE BOVINE PH-20, ON THE SPERM PLASMA AND ACROSOME MEMBRANE**
Guillaume Morin, PhD, Laval University, Robert Sullivan, PhD, Laval University and Pierre Leclerc, PhD, Laval University (Presented By: Guillaume Morin, PhD, Laval University)

MALE SEXUAL FUNCTION/PROSTATE/TESTIS CANCER/CLINICAL UROLOGY

Poster# 50 **TREATMENT TO A HARDNESS GOAL OF FULL RIGIDITY IN MEN WITH ERECTILE DYSFUNCTION IS ASSOCIATED WITH SIGNIFICANT IMPROVEMENTS IN EMOTIONAL WELL-BEING AND SATISFACTION**
Gerald Brock, MD FRCS(C), St. Joseph's Health Care Center, Irwin Goldstein, MD, The Journal of Sexual Medicine, John P. Mulhall, MD, Weill Medical College of Cornell University, Ivan Levinson, FCS, Pfizer Inc and Dana Creanga, PhD, Pfizer Inc (Presented By: Gerald Brock, MD FRCS(C), St. Joseph's Health Care Center)

Poster# 51 **6 MONTH INTERIM ANALYSIS OF THE LONGITUDINAL EFFECTS ON PENILE OXYGEN SATURATION FROM A RANDOMIZED STUDY OF THE NIGHTLY USE OF INTRAURETHRAL ALPROSTADIL VS SILDENAFIL FOLLOWING NERVE SPARING RADICAL PROSTATECTOMY (NSRRP)**
David Fenig, MD, New York University School of Medicine, David Robbins, MD, New York University School of Medicine, Donna Brassil, RN, New York University School of Medicine, Brianne Goodwin, RN, New York University School of Medicine and Andrew McCullough, MD, New York University School of Medicine (Presented By: David Fenig, MD, New York University School of Medicine)

Poster# 52 **PENILE PROSTHETIC INFECTIONS IN PATIENTS UNDERGOING MULTIPLE PROSTHETIC-RELATED OPERATIONS**
Kamran Sajadi, MD, Section of Urology, Medical College of Georgia and Ronald Lewis, MD, Section of Urology, Medical College of Georgia (Presented By: Kamran Sajadi, MD, Section of Urology, Medical College of Georgia)

Poster# 53 **CLINICAL SAFETY, HISTOLOGIC FINDINGS AND SURGICAL METHODS IN COMPLEX PHALLOPLASTY USING TYPE I COLLAGEN**
Joon Yong Kim, MD, PhD, Manomedi Clinics for Andrology and Urology, Hoon Seog Jeon, Beom Joon Kim, Professor, MD, Dongguk Medical College and Kye Yong Song, Professor, MD, Joongang Medical College (Presented By: Joon Yong Kim, MD, PhD, Manomedi Clinics for Andrology and Urology)

Poster# 54 **ADMINISTRATION OF PHOSPHODIESTERASE 5 INHIBITORS IN MEN WITH NON-INFLAMMATORY CHRONIC PELVIC PAIN**
Dimitrios Baltogiannis, MD, PhD, Ioannina University School of Medicine, Department of Urology, Greece, Nikolaos Giotitsas, BS, Ioannina University School of Medicine, Department of Urology, Ioannina, Greece, Dimitrios Giannakis, MD, PhD, Ioannina University School of Medicine, Department of Urology, Ioannina, Greece, Nikolaos Pardalidis, MD, PhD, Ioannina University School of Medicine, Department of Urology, Ioannina, Greece, Ikuo Miyagawa, MD, PhD, Tottori University School of Medicine, Department of Urology, Yonago, Japan and Nikolaos Sofikitis,MD, PhD, DMSci, Ioannina University School of Medicine, Department of Urology, Ioannina, Greece (Presented By: Dimitrios Baltogiannis, MD, PhD, Ioannina University School of Medicine, Department of Urology, Greece)

Poster# 55 **EFFECTS OF VARDENAFIL ON SPERM PARAMETERS AND SEMEN BIOCHEMISTRY**
Evagelos Grammeniatis, MD, Department of Urology, Ioannina University School of Medicine, Nikolaos Kanakas, MD, PhD, Department of Urology, Ioannina University School of Medicine, Panagiota Tsounapi, BS, Department of Urology, Ioannina University School of Medicine, Dimitrios Baltogiannis, MD, PhD, Department of Urology, Ioannina University School of Medicine, Ikuo Miyagawa, MD, PhD, Department of Urology, Tottori University School of Medicine and Nikoalos Sofikitis, MD, PhD, Department of Urology, Ioannina University School of Medicine (Presented By: Evagelos Grammeniatis, MD, Department of Urology, Ioannina University School of Medicine)

Poster# 56 **CYTOKINES MCP-1, MIF AND ENA-78 IN SEMINAL PLASMA: RELATIONSHIPS WITH INFLAMMATION AND CHRONIC PELVIC PAIN SYNDROME**
Krister Freese, BA, Univ of Washington, Charles Muller, PhD, University of Washington, Erin Pagel, BS, Univ of Washington, Claire Yang, MD, Univ of Washington and Richard Berger, MD, Univ of Washington (Presented By: Charles Muller, PhD, University of Washington)

EPIDIDYMIS

Poster# 57 **CHARACTERIZING THE BIOLOGICAL FUNCTION OF CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PROTEIN USING A KNOCKOUT MOUSE MODEL**
Kim Chau, BS, Texas Tech University Health Sciences Center and Gail Cornwall, PhD, Texas Tech University Health Sciences Center (Presented By: Kim Chau, BS, Texas Tech University Health Sciences Center)

Poster# 58 **INVOLVEMENT OF ANGIOTENSIN II TYPE 2 RECEPTOR (AT2) IN VACUOLAR H+-ATPASE (V-ATPASE) APICAL INSERTION AND V-ATPASE-DEPENDENT PROTON SECRETION IN EPIDIDYMAL CLEAR CELLS**
Winnie W.C. Shum, PhD, Massachusetts General Hospital - Harvard Medical School, Nicolas Da Silva, PhD, Massachusetts General Hospital - Harvard Medical School, Richard Bouley, PhD, Massachusetts General Hospital - Harvard Medical School, Jaafar El Annan, MD, Massachusetts General Hospital - Harvard Medical School, Peter J.S. Smith, PhD, Biocurrents Research Center - Marine Biological Laboratory and Sylvie Breton, PhD, Massachusetts General Hospital - Harvard Medical School (Presented By: Winnie W.C. Shum, PhD, Massachusetts General Hospital - Harvard Medical School)

Poster# 59 **ANDROGEN REGULATION OF APOPTOSIS AND SURVIVAL IN THE EPIDIDYMIS**
Sophie-Anne Lamour, BSc, McGill University and Bernard Robaire, BSc; PhD, McGill University (Presented By: Sophie-Anne Lamour, BSc, McGill University)

Poster# 60 **NEW INSIGHTS IN SPERM ACQUISITION OF GPI-LINKED PROTEINS DURING EPIDIDYMAL MATURATION AND CAPACITATION: A ROLE FOR CLUSTERIN/APOJ**
Genevieve Griffiths, BS, University of Delaware and Patricia Martin-DeLeon, PhD, U. Delaware (Presented By: Genevieve Griffiths, BS, University of Delaware)

Poster# 61 **CRES (CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC) PROTEIN** Seethal Johnson, Texas Tech University Health Sciences Center, Douglas Swartz, BS Biochemistry, TTUHSC, Kim Chau, BS Biology, TTUHSC, Hans Henning von Horsten, PhD Reprod Biol, TTUHSC, Sandra Whelly, PhD Biochemistry, TTUHSC and Gail Cornwall, PhD Reprod Biol, TTUHSC (Presented By: Seethal Johnson, Texas Tech University Health Sciences Center)

poster session 2

Monday, April 23, 2007
10:45 a.m. 12:30 p.m.
Location: Regency Ballroom 1-3

ANDROGENS/ENDOCRINOLOGY

Poster# 62 **TESTOSTERONE PRODUCTION OF ISOLATED LEYDIG CELLS IN XXY MICE**
Monica Schwarcz, MD, LA BioMed at Harbor-UCLA Medical Center, YanHe Lue, MD, Harbor-UCLA Medical Center, Christina Wang, MD, Harbor-UCLA Medical Center and Ronald Swerdloff, MD, Harbor-UCLA Medical Center (Presented By: Monica Schwarcz, MD, LA BioMed at Harbor-UCLA Medical Center)

Poster# 63 **IDENTIFICATION OF GALANIN RECEPTOR 2 IN RAT LEYDIG CELLS**
Renshan Ge, MD, MS, Population Council, Han Lin, PHD , MD, Wenzhou Medical College, Guorong Chen, MD, Wenzhou Medical College, Chantal Sottas, BA, Population Council and Matthew Hardy, PHD, Population Council (Presented By: Renshan Ge, MD, MS, Population Council)

Poster# 64 **ENCLOMIPHENE (ANDROXAL™) RAISES TESTOSTERONE IN MEN WITH LOW OR NORMAL TESTOSTERONE AND PROVIDES A MORE NORMAL DAILY RHYTHM**
Ronald Wiehle, PhD, Repros Therapeutics Inc and Joseph Podolski, BS, Repros Therapeutics, Inc. (Presented By: Ronald Wiehle, PhD, Repros Therapeutics Inc)

SPERMATOGENESIS/STEROIDOGENESIS/TESTIS BIOLOGY

Poster# 65 **11ß-HYDROXYSTEROID DEHYDROGENASE (11ß-HSD) ISOZYMES IN THE HUMAN TESTIS**
Cigdem Tanrikut, MD, New York-Presbyterian Hospital/Weill Cornell Medical College, Dianne Hardy, PhD, Population Council, Renshan Ge, MD, Population Council, Chantal Sottas, BA, Population Council, James Catterall, PhD, Population Council, Darius Paduch, MD, PhD, New York-Presbyterian Hospital/Weill Cornell Medical College, Population Council, Marc Goldstein, MD, New York-Presbyterian Hospital/Weill Cornell Medical College, Population Council, Peter Schlegel, MD, New York-Presbyterian Hospital/Weill Cornell Medical College, Population Council and Matthew Hardy, PhD, Population Council (Presented By: Cigdem Tanrikut, MD, New York-Presbyterian

Poster# 66 **ELMO INCREASES ENGULFMENT IN SERTOLI CELLS**
Robin Woodson, BA, Department of Urology University of Virginia, Shuqiu Zheng, PhD, Department of Urology University of Virginia and Jeffrey Lysiak, PhD, Department of Urology University of Virginia (Presented By: Jeffrey Lysiak, PhD, Department of Urology University of Virginia)

Poster# 67 **ENHANCED CAMP-RESPONSIVE ELEMENT MODULATOR (CREM) IN RAT TESTES TREATED BY GINSENG RADIX**
Won-Nam Kim, BA, Dept. of Prescriptionology, Dong Youp Shin, PhD, Woong Mo Yang, MS, Wansu Park, PhD, Mun Seog Chang, PhD, Jun Bok Jang, PhD and Seong Kyu Park, PhD (Presented By: Won-Nam Kim, BA, Dept. of Prescriptionology)

Poster# 68 **YUKMIJIHWWANG-TANG AN ENHANCEMENT OF CREM: ON REDUCED SPERMATOGENESIS BY CYCLOPHOSPHAMIDE**
Eun-Hwa Park, BA, Dept. of Prescriptionology, Myung Sook Oh, PhD, Do Rim Kim, MS, Dong Gi Choi, MS, Dong Min Kim, MS, Woong Mo Yang, MS, Nam-IL Kim, PhD and Seong Kyu Park, PhD (Presented By: Eun-Hwa Park, BA, Dept. of Prescriptionology)

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SUNDAY, APRIL 22, 2007
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session I:
Spermatogenesis, Infertility,
Contraception and Sexual Function
Moderators: John K. Amory, MD and Michaela Luconi, PhD
Location: Regency 4-7

1

mRNA PROFILES IN SPERM OF PATIENTS WITH PROTAMINE DEREGULATION INDICATE A MORE GENERALIZED DEVELOPMENTAL PATHOLOGY

Benjamin R. Emery[1], BS, Stephen A. Krawetz[2], PhD, Adrian E. Platts[2], BS, Douglas T. Carrell[1], PhD,
Andrology & IVF Laboratories, University of Utah School of Medicine[1]. Department of OB-GYN, Wayne State University School of Medicine[2]

The mature sperm is transcriptionally quiescent, but contains a suite of mRNAs that are retained from earlier stages of spermatogenesis. These transcript profiles are consistent within fertile men. Interestingly, there is variance observed within infertile individuals, which may be of diagnostic significance. Previous work has also identified there is variation in the number of protamine 1 (P1) and protamine 2 (P2) transcripts present in infertile men with protamine deregulation.
We have analyzed mRNA transcript profiles from ejaculated sperm in two cohorts of men. One with protamine protein deregulation and P1 transcript retention (6) and a second group of fertile donors (8) using the Illumina Sentrix 6: Human Whole Genome BeadChip arrays. Briefly, sperm were collected from men under IRB approval. Mature sperm mRNA was isolated following a stringent somatic cell lysis protocol and subjected to two rounds of amplification using the MessageAmp II aRNA Amplification Kit and labeled for chip hybridization. The Illumina chips were processed and quantified following standardized laboratory procedures.
These data were subjected to hierarchal clustering and show that men with protamine deregulation have an altered retention of spermatogenesis-related transcripts when compared to donors of known fertility. Furthermore, samples were subjected to supervised hierarchal clustering, based on a subdivision of spermatogenesis-related genes into two groups, those required for late stages of spermatogenesis and bound into mRNP particles by MSY2 in the mouse (presumed to be bound by it's human homlouge, contrin) and those non-bound. This clustering shows a pattern of similarity within the patient group, which segregates from donors of known fertility.
This analysis indicates that protamine deregulation is associated with a more general dysfunction of spermatogenesis and that there is some indication contrin-bound transcripts are more variable than non-bound transcripts in the abnormal protamine expression group.

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DISULFIDE BONDS OF SPERMATOGENIC CELL-SPECIFIC TYPE 1 HEXOKINASE (HK1S) AND SPERM MOTILITY

Noriko Nakamura, PhD, and Edward M. Eddy, PhD
National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC

We identified previously three spermatogenic cell-specific mRNA splice variants for type 1 hexokinase with different 5' untranslated regions. All encode the same protein (HK1S) with a novel N-terminal peptide sequence, the spermatogenic cell-specific region (SSR). The HK activity in undiluted cauda epididymal (quiescent) sperm is significantly lower than in actively motile sperm 5 min after dilution in M2 medium. We found this change (sperm activation) is associated with HK1S disulfide bond reduction and dephosphorylation. Treatment with diamide (inhibitor of disulfide bond reduction) blocked dephosphorylation of HK1S and sperm activation. This suggested disulfide bond reduction is necessary for dephosphorylation, the increase in HK activity and initiation of motility. Therefore, we examined the effects of diamide and okadaic acid (OA) (phosphatase inhibitor) on HK activity and sperm motility. Treatment with 1 or 10 mM diamide inhibited HK activity and treatment with 10 mM diamide inhibited sperm motility. However, treatment with OA did not inhibit HK activity or sperm motility. To determine if the effect was specific or due to toxicity, ATP levels of sperm incubation in M2 medium with 1 or 10 mM diamide were measured after 5 min and found to be not different from untreated sperm. There also was no difference in ATP levels in sperm treated with 1 mM or 10 mM of OA. These results provide further support for the concept that HK1S disulfide bond reduction precedes dephosphorylation and initiation of motility of sperm from the cauda epididymis. Furthermore, an antibody to phosphotyrosine recognized a dimer in quiescent caput and cauda epididymal sperm that was not detected in activated caput or cauda sperm. An antibody to the SSR region of HK1S recognized a dimer band in quiescent cauda sperm and a single band in activated cauda sperm, but recognized a dimer in both quiescent and activated caput sperm. Since motility of activated caput sperm is much weaker than that of activated cauda sperm, this suggests that the increase in HK activity and sperm motility are associated with a conversion of at least some HK1S from a dimeric to a monomeric state.

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IMMUNE REGULATION IN HUMAN TESTIS

Maciej Kurpisz, Dorota Fiszer, Natalia Rozwadowska, Piotr Jedrzejczak[1], W[3]odzimierz Kosicki[2]
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Introduction: Spermatogenesis appears as the particularly complex process of cell differentiation. Growth and differentiation of gametogenic cells is supported by the somatic ones from which signals of proliferation and apoptosis keep the appropriate balance until the final conversion into spermatozoa. Inside of seminiferous epithelium a unique composition of the a) histocompatibility antigens, b) cytokines, c) apoptotic factors, creates the semi-privileged immunological zone.
Objective: To investigate quantitative level of selected gene expression belonging to HLA and IL-1-IL-18 systems.
Methods: Complex studies using real-time PCR, Northern blotting and other molecular techniques of the range of genes belonging to the HLA system as well as to IL-1-IL-18 family. There have been studied samples of homogenated tissue samples originated from normal testis, spermatogenetic arrests and testicular tumors.
Results: Histocompatibility genes expression is uniquely composed within seminiferous epithelium consisting of inversely related levels of classical HLA Class Ia and non-classical HLA Class Ib genes (HLA-E and –F). The latter ones were mostly located in adluminal compartment. Studied IL-1-IL-18 superfamily revealed compartmentalization between intratubular and interstitial compartments with IL-1 beta prevalence in the interstitium and IL-1 alpha in seminiferous tubules. IL-18 seems to be low expressed in physiological testis while it is increased in malignancy. A proportion between IL-1 alpha to IL-1RA seems to be a crucial factor for the proper differentiation of gametogenic cells.
Conclusions: It appears that molecular factors and their level of transcription can be a strong indicator for the determination of the spermatogenetic process. Genes of IL-1-IL-18 superfamily may constitute important prognostic factors that can be used for future diagnostic purposes.

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ETIOLOGY OF SEMINIFEROUS TUBULE BASEMENT MEMBRANE THICKENING (BMT) IN INFERTILE MEN WITH VARICOCELES

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Introduction: BMT with varicoceles is progressive, increasing with age. We previously reported that as BMT increased, the circumference of the lumen of the seminiferous epithelium decreased, leading to increased apoptosis and to an overall decrease in the number of developing germ cells. However, the mechanism(s) underlying this process remained elusive.
Objectives: To determine the relationship between BMT and other parameters regulating apoptosis and infertility with varicoceles (e.g., damaging: elevated testicular cadmium [Cd], Fas, iNOS; protective: VEGF, heat shock protein 70-1 [HSP70-1], cytochrome P450 side chain cleavage enzyme [P450scc]).
Methods: Testis biopsies were obtained from 40 patients with varicoceles and 3 controls with obstructive azoospermia. BMT was measured with a Vernier ocular on 5 tubules/patient. Total counts/tubule included spermatogonia, spermatocytes, round spermatids and spermatozoa. Preoperative sperm counts were assessed on a hemacytometer. Cd was quantified by atomic absorption and apoptosis by TUNEL. HSP 70-1 mRNA was detected by RT-PCR against constitutively expressed HSP70-HOM control. P450ssc, VEGF, Fas and iNOS antibody staining were scored by two blinded observers on a scale of 0-4. Data were analyzed by Spearman correlations (SC) and Mann-Whitney (MW) tests.
Results: BMT was higher in patients with bilateral varicoceles than left varicoceles (MW, $P<0.02$). BMT was positively correlated with iNOS (SC, $n=25$, $r=0.42$, $P<0.04$) and negatively related to VEGF (SC, $n=19$, $r= -0.57$, $P<0.01$), P450scc (SC, $n=21$, $r= -0.44$, $P<0.5$) and HSP70-1 (MW, $P<0.03$). VEGF was positively associated with number of spermatogonia (SC, $n=19$, $r=0.48$, $P<0.04$) and spermatozoa (SC, $r=0.53$, $P<0.02$). The preoperative sperm count was related to the number of spermatogonia (SC, $n=20$, $r=0.53$, $P<0.02$). Cd was positively correlated with iNOS (SC, $n=37$, $r=0.54$, $P<0.0001$) and VEGF (SC, $n=15$, $r=0.50$, $P<0.05$). iNOS was positively related to Fas (SC, $n=12$, $r=0.74$, $P<0.005$) and germ cell apoptosis (SC, $n=39$, $r=0.82$, $P<0.0001$).
Conclusions: BMT was positively related the number of varicoceles and oxidative stress (iNOS), and negatively influenced by VEGF, P450scc and HSP70-1. As in animal models, VEGF regulated both spermatogenesis and spermiogenesis. iNOS was induced by elevated testicular Cd levels and influenced the level of germ cell apoptosis directly through the death receptor pathway and indirectly via induction of BMT. Thus, BMT is a complex function of both intrinsic and extrinsic factors promoting varicocele-associated infertility.
Support: PHS Grant No. ES10496 to SB.

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SIMILAR INTRATESTICULAR ANDROGEN CONCENTRATIONS IN MEN WITH SEVERE OLIGOSPERMIA VERSUS THOSE WITH POORLY SUPPRESSED SPERMATOGENESIS INDUCED BY A MALE HORMONAL CONTRACEPTIVE REGIMEN

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Background: Male hormonal contraceptive regimens block spermatogenesis by inhibiting gonadotropin secretion, leading to a dramatic decrease in testicular androgen biosynthesis. Animal studies suggest there is a direct relationship between persistent intratesticular androgen production and

spermatogenesis. Therefore, we hypothesized that men with more poorly suppressed spermatogenesis after prolonged male hormonal contraceptive treatment would have higher intratesticular androgen concentrations than those men who achieved severe oligospermia.
Methods: Healthy men, ages 25-55 years, were randomized to treatment for 24 wks with either 1) transdermal testosterone (T) gel (100 mg daily) + depomedroxyprogesterone acetate (DMPA, 300 mg IM/12 wks) or 2) a GnRH antagonist, acyline (300 µg/kg SC/2 weeks x 12 wks) + T gel + DMPA. Testicular fine needle aspirations of both testes were performed in 19 men during the last week of treatment. Intratesticular T (*it*-T) and dihydrotestosterone (*it*-DHT) were measured by liquid chromatography-mass spectrometry.
Results: All men had dramatic suppression of spermatogenesis; 14/19 men were severely oligospermic (sperm concentration d"1 million/ml) or azoospermic, and 5/19 were poorly suppressed to 1.6-3.2 million sperm/ml. Mean *it*-T and *it*-DHT concentrations were 37±8 and 5.5±0.8 nmol/L, respectively, after treatment, similar to values reported after shorter term gonadotropin suppression and measured by immunoassay. Intratesticular androgen concentrations were not significantly different in men who did and did not achieve severe oligospermia (p=0.56 for *it*-T and p=0.14 for *it*-DHT). Furthermore, there was no significant correlation between *it*-T or *it*-DHT levels, and sperm concentrations or gonadotropins before or after male hormonal contraceptive treatment.
Conclusions: In this study of prolonged gonadotropin withdrawal induced by male hormonal contraceptive treatment, we failed to demonstrate a relationship between persistent spermatogenesis and intratesticular androgen concentrations. Additional studies to identify factors involved in human spermatogenesis in the low-androgen testicular environment are warranted.

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UROSELECTIVE ALPHA-1 BLOCKER ALFUZOSIN RESTORED ERECTILE DYSFUNCTION AFTER PARTIAL BLADDER OUTLET OBSTRUCTION IN RATS

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Introduction and Objectives: Benign prostate hyperplasia (BPH) /lower urinary tract symptoms (LUTS) and male sexual dysfunction are highly prevalent in ageing men. Alfuzosin is a uroselective alpha-adrenoceptor antagonist commonly used to treat BPH/LUTS. The specific aims of our study were (a) to determine whether the development of LUTS in rat bladder following partial bladder outlet obstruction (PBOO) affects erectile tissue function, and (b) if alfuzosin can relieve PBOO and improve ED.
Methods: Male Sprague-Dawley rats were randomized into 4 groups: (1) control; (2) alfuzosin-treated control; (3) PBOO rats; and (4) alfuzosin-treated PBOO rats. Groups (3) and (4) were subjected to PBOO for 6 weeks by suture ligation. Rats in groups (2) and (4) received oral administration of alfuzosin (10 mg/kg) for 6 weeks. Erectile response was measured) using our established rat model. Isolated CC strips were dissected from all rats and mounted for force generation measurement during organ bath studies. Contractile responses to phenylephrine (Phe), and relaxant responses to acetylcholine (ACh) and endothelium-independent relaxation to sodium nitroprusside (SNP) were studied.
Results: All PBOO animals showed 80% decreased ICP/MAP ratios and 70% decrease in total ICP values at 7.5V compared to controls. Alfuzosin (10 mg/kg dose) in PBOO animals demonstrated significant improvement in ICP/MAP and total ICP values. In in vitro studies, the maximum nitrergic nerve-mediated relaxation of Phe-induced precontracted cavernosum in PBOO group was 67% reduced ($p<0.001$). Alfuzosin treatment normalized such decline in isolated CC strips. Endothelium-independent relaxation to SNP was not altered by alfuzosin in rats with PBOO.
Conclusions: Our study showed that Alfuzosin treatment following PBOO improves erectile function probably mediated via neuronal- and endothelial-NO relaxant expression. Thus, selective inhibition of alpha-1 receptors in the penis can correct NO-dependent PBOO-induced ED.

(Sponsored by: Sanofi-Aventis)

SUNDAY, APRIL 22, 2007
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session II
Sperm Function, ART, Epididymis, and Fertilization
Moderators: Patricia A. Martin-DeLeon, PhD and Genevieve Griffiths, BA
Location: Garrison Suite

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COMPLEMENTARY STUDY OF SPERM CHROMATIN QUALITY IN CANCER PATIENTS BY FLOW CYTOMETRY
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Testis cancer (TC) and Hodgkin's lymphoma (HL) are common malignancies affecting men in their reproductive age that can be managed successfully by chemotherapy. Sperm cryopreservation pre-chemotherapy remains the only feasible way to preserve their fertility. The objective of the current study was to evaluate sperm chromatin quality in cancer patients prior to chemotherapy when sperm cryopreservation takes place. A cohort of subjects (age 21-48 yrs) with advanced TC (n=12) and HL (n=11) and idiopathic infertility (IF) (n=21) were recruited along with 19 healthy community volunteers (CV). Semen parameters were analyzed according to the WHO criteria. Sperm chromatin quality was determined by using four complementary tests: the acridine orange (AO)/sperm chromatin structure assay (SCSA®), terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL), chromomycin A3 (CMA3) and monobromobimane thiol (SH) labeling (mBBr) assays using flow cytometry. A higher sperm chromatin quality was noted in TC and HL than IF patients using DNA fragmentation index (DFI) by AO, TUNEL and mBBR assays ($p<0.05$). However, CMA3 or high DNA stainability (HDS) by AO failed to demonstrate a difference among groups. In addition, TC and HL groups have higher sperm concentration and lower percentage of morphologically abnormal spermatozoa (MAS) than in IF ($p<0.05$). Progressive motility (PM) was lower in IF and in TC, but not in HL ($p<0.05$). There was a negative correlation among PM and DFI, TUNEL and SH ($p<0.05$). Sperm concentration did not correlate with DFI or SH, but did correlate with TUNEL ($p<0.05$). MAS correlated with DFI, TUNEL and SH ($p<0,05$). Although, we found 22%, 14% and 27% of asthenospermic samples (from IF, TC and HL) with low DFI, TUNEL and high SH values respectively. Moreover, there were 11%, 1% and 3% of samples with low MAS but with high DFI, TUNEL and SH respectively. In conclusion, parameters from routine semen analysis do not absolutely predict sperm chromatin quality of human semen samples. The complementary use of flow cytometry-based assays could be a useful tool to determine sperm chromatin quality in sperm samples submitted for cryopreservation before chemotherapy in cancer patients.

Supported by CIHR.

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NYD-SP27, AN INTRINSIC DECAPACITATION FACTOR IN SPERM
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Prior to fertilization sperm have to undergo an activation process known as capaciation, leading to the acrosome reaction, which releases hydrolytic enzymes to facilitate sperm penetration of the egg. However, premature capacitation may lead to untimely release of sperm acrosomal enzymes before they reach the egg. Little is known about the mechanism for preventing premature capacitation in sperm although decapacitation factors from various sources have been thought to be involved.
Objectives: To investigate the role and mechanism of NYD-SP27 in sperm, which is an isoform of phospholipase C (PLC)æ , previously shown to exert an inhibitory effect on the PLC-coupled Ca2+ mobilization in the pancreas.
Methods: We examin the location of NYD-SP27 in sperm by immunofluorescence. CTC and immunofluorescence double staining show that NYD-SP27 is detached from the sperm during capacitation and acrosome reaction. We demonstrate that the specific antibody to NYD-SP27 could block the detachment of NYD-SP27, use the antibody and PLC inhibitor to study the effect on capacitation and acrosome reaction. Also the $[Ca^{2+}]_i$ in sperm is examined to clarify the underlying mechanism.
Results: NYD-SP27 is localized to sperm acrosome and shown to be detached from sperm as they undergo capacitation and acrosome reaction. The absence of HCO3-, a key factor in activating capacitation, from the capacitation-inducing medium prevents the lost of NYD-SP27 from sperm. The antibody against NYD-SP27 also prevents the lost of NYD-SP27 from sperm and significantly reduces the number of capacitated sperm and acrosome reaction induced by ATP and progesterone, as well as inhibits the PLC-coupled Ca2+ mobilization in sperm, which can be mimicked by an inhibitor of PLC, U73122.
Conclusions: These data strongly suggest that NYD-SP27 is a physiological inhibitor of PLC that acts as an intrinsic decapacitation factor in sperm to prevent premature capacitation and acrosome reaction.

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IMPROVED SPERM CHROMATIN DECONDENSATION RATES IN HAMSTER-OOCYTE ICSI AFTER ANNEXIN-V-MACS IN INFERTILITY PATIENTS

Sonja Grunewald[1], Verona Blumenauer[2], Martin Reinhardt[1], Ashok Agarwal[3], Tamer M. Said[4], Fayez Abu Hmeidan[2], Hans-Juergen Glander[1], Uwe Paasch[1].
[1]EAA Center, University of Leipzig, Germany, [2]Clinic of Reproductive Medicine, Leipzig, Germany, [3]Reproductive Research Center, Cleveland Clinic Foundation, Cleveland, USA, [4]Toronto Institute of Reproductive Medicine, Toronto, Canada.

The depletion of apoptotic sperm using Annexin-V based magnetic cell separation (MACS) has been recently introduced as a mean to improve assisted reproduction outcomes. Annexin-V MACS enhances hamster-oocyte sperm penetration but does not increase sperm chromatin decondensation (SCD) rates following hamster-oocyte ICSI (H-ICSI). Our aim was to evaluate the SCD rates of the annexin-negative (non-apoptotic) sperm fraction of patients with infertility using H-ICSI.
Semen specimens collected from 21 infertility patients with subnormal sperm parameters were subjected to double density gradient centrifugation (DGC) followed by Annexin-V MACS. A non-separated aliquot of each sample served as control. H-ICSI was performed in all aliquots using 20 frozen-thawed hamster oocytes per aliquot. Results were evaluated as the percentage of oocytes showing SCD. In addition, caspase-3 activation (CP3) and disruption of transmembrane mitochondrial potential (TMP) were monitored by FACS to observe the separation effect.
Annexin-V MACS resulted in a significant enrichment of spermatozoa with inactive CP3 and intact TMP in the annexin-negative fraction. Similarly, annexin-negative spermatozoa had the highest SCD rates compared to controls and annexin-positive sperm (table).
In conclusion, semen samples from infertility patients contain high levels of spermatozoa with active CP3 and disrupted TMP. Compared to routine DGC the enrichment of non-apoptotic spermatozoa by Annexin-V MACS resulted in superior sperm chromatin decondensation and may be used to improve the outcome of ICSI procedures in infertility patients.

Parameter	Controls	Annexin-negative	Annexin-positive
active CP3 (% sperm)	43.5 ± 13.8	26.8 ± 12.3*	58.4 ±11.7*
intact TMP (% sperm)	54.7 ± 23.2	71.6 ± 21.5*	9.8 ±12.0*
SCD (% oocytes)	31.3 ± 13.1	44.2 ± 15.8*	18.3 ± 6.7*

Values are expressed as mean ± standard deviation. *$p<0.01$ in comparison to control. Statistical test: Wilcoxon-test.

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MULTIPLE STEPS IN THE FERTILIZATION CASCADE ARE IMPAIRED WHEN SPERM GLYCOLYSIS IS DISRUPTED

Zaohua Huang[1], Kathleen Mohr[2], Deborah A. O'Brien[1]
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Glyceraldehyde 3-phosphate dehydrogenase-S (GAPDHS) is a sperm-specific glycolytic enzyme in mammals. Male mice lacking this enzyme are infertile and produce sperm with very low ATP levels and severe motility defects (Miki *et al.*, 2004; PNAS 101:16501). We examined multiple steps in the fertilization cascade to further assess the functional competence of sperm lacking this enzyme. GAPDHS-null sperm did not exhibit tyrosine phosphorylation when incubated in capacitation medium for 2 h. The addition of a potent, cell-permeable cAMP analog (0.1 mM cBiMPS) and a phosphodiesterase inhibitor (0.1 mM IBMX) induced tyrosine phosphorylation in wild-type sperm, but not in GAPDHS-null sperm. These results indicate that glycolysis is required for capacitation-dependent tyrosine phosphorylation, even under conditions that elevate intracellular cAMP levels. GAPDHS-null sperm also have significantly reduced zona binding capacity. In parallel binding assays we typically observed less than 10 GAPDS-null sperm/oocyte compared to more than 25 wild-type sperm/oocyte. In contrast, no defects in induction of the acrosome reaction were detected. Similar percentages of wild-type and GAPDHS-null sperm were acrosome reacted following incubation with calcium ionophore (53-57%) or heat-solubilized zonae pellucidae (46-47%). Compared to *in vitro* fertilization (IVF) rates >50% with sperm from wild-type males, GAPDHS-null sperm rarely achieved IVF and oocytes that appeared to be fertilized never developed beyond the 2-cell stage. We also compared IVF rates after zona drilling using a laser ablator, which increased the fertilization rate with GAPDHS-null sperm to 30.3%. In these experiments, development to the blastocyst stage was indistinguishable following fertilization with GAPDS-null or wild-type sperm. The above studies confirm the critical roles of GAPDHS and glycolysis in multiple steps of fertilization and suggest that sperm-specific glycolytic enzymes may be excellent targets for contraceptive development.

Supported by NICHD/NIH through cooperative agreement U54 HD35041 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research.

11

PROTEOMIC ANALYSIS OF EPIDIDYMAL CLEAR CELLS AND V-ATPase-ASSOCIATED PROTEINS

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An acidic luminal pH in the epididymis, and vas deferens, contributes to maintaining sperm in a quiescent state during their maturation and storage. We have shown that clear and narrow cells, which are members of the "mitochondria-rich" cell family, express high levels of the vacuolar H^+-ATPase (V-ATPase) in their apical membrane and are major contributors to luminal acidification. Our current work is aimed at characterizing the proteomic profile of narrow/clear cells and of V-ATPase interacting proteins.
Total proteomic analysis was first performed on EGFP-expressing clear cells isolated by fluorescence activated cell sorting (FACS), from the epididymides of our B1-EGFP transgenic mice (Miller et al., Am J Physiol Cell Physiol. 2005). In these mice, EGFP expression, driven by the promoter of the V-ATPase B1 subunit, occurs specifically in clear cells. A 98% pure population of clear/narrow cells was generated and a total of 450 proteins were identified by mass spectrometry (LC-MS/MS) in these cells. These included several V-ATPase subunits such as the clear cell-specific a4, d1 and B1 subunits, and mitochondrial proteins consistent with the abundance of the V-ATPase and mitochondria in these cells. Several cytoskeletal proteins, including actin, tubulin, and actin regulating proteins such as gelsolin were also detected, in agreement with the dynamic cytoskeletal remodeling that occurs in these cells. A more detailed proteomic analysis was performed from epididymal proteins co-immunoprecipitated with the B1 subunit. As expected, B1 was detected together with other V-ATPase subunits in this sample. Interestingly, several proteins of the myosin and kinesin family were identified together with actin and tubulin and accounted for about 20 % of total immunoprecipitated proteins. The actin regulatory proteins, gelsolin and drebrin, and the RhoGAP nadrin, which regulates exocytosis via reorganization of cortical actin, were also found. The presence of a complex of actin-regulatory proteins associated with the V-ATPase indicates that the V-ATPase itself can participate in the management of vesicle trafficking via local actin remodeling in clear cells.

This work is supported by NIH grants HD40793, DK38452 and DK42956.

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EVIDENCE FOR THE INVOLVEMENT OF TESTICULAR PROTEIN CRISP2 IN MOUSE SPERM-EGG FUSION

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Epididymal protein DE and testicular protein Tpx-1 are two cysteine-rich secretory proteins known as CRISP1 and CRISP2, respectively. Rat CRISP1 (rCRISP1) is localized in the equatorial segment of acrosome-reacted sperm and participates in gamete fusion through its binding to egg-complementary sites. Recent results using bacterially-expressed fragments of rCRISP1 as well as synthetic peptides revealed that the ability of rCRISP1 to bind to the egg surface resides in a region of 12 amino acids corresponding to an evolutionarily conserved motif of the CRISP family named Signature 2. Interestingly, mouse CRISP2 (mCRISP2) exhibits only two substitutions in Signature 2 when compared to rCRISP1, and it is also capable of binding to the rat egg, opening the possibility for a role of CRISP2 in gamete fusion. In view of this, in the present work we have used a mouse model to examine both the sub-cellular localization of CRISP2 in sperm and its involvement in fertilization. Results from indirect immunofluorescence and protein extraction experiments support that mCRISP2 is an intra-acrosomal component that remains associated with sperm after capacitation and acrosome reaction. In vitro fertilization assays using zona pellucida-intact eggs show that a polyclonal antibody against the protein significantly decreases the percentage of penetrated eggs with a concomitant accumulation of perivitelline sperm. The failure to inhibit zona pellucida penetration excludes a detrimental effect of the antibody on sperm motility and/or acrosome reaction supporting a specific participation of mCRISP2 at the sperm-egg fusion level. In agreement with this, recombinant mCRISP2 (recCRISP2) specifically binds to the fusogenic area of mouse eggs as previously reported for rCRISP1. The similar localization of CRISP1- and CRISP2-binding sites on the mouse egg, and the high homology of the corresponding Signature 2 regions, suggest that both proteins might interact with the same egg binding sites. In vitro competition studies show that incubation of zona-free eggs with a fixed concentration of recCRISP2 and increasing amounts of rCRISP1, reduce the binding of recCRISP2 to the egg suggesting that the two proteins interact with common egg complementary sites. These results provide evidence for the involvement of both epididymal CRISP1 and testicular CRISP2 in gamete fusion, supporting the idea of a functional cooperation between homologous molecules as a mechanism to ensure the success of fertilization.

SUNDAY, APRIL 22, 2007
10:45 a.m. – 12:30 p.m.

Poster Session I
Location: Regency Ballroom 1-3

ANDROGENS/ENDOCRINOLOGY

13

INVOLVEMENT OF JNK AND P38 MAP KINASE IN CARDIOTOXIN-INDUCED MUSCLE INJURY: ROLE OF TESTOSTERONE

Indrani Sinha-Hikin, Ruoqing Shen, Benjamine Tran, Mellisa Braga
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Background: Apoptosis has been implicated as a mechanism of muscle cell death during skeletal muscle development, in aging, and can be triggered by various injuries. The signaling events leading to apoptosis can be divided into two pathways, involving either mitochondria (intrinsic) or death receptors (extrinsic). Recently, using a well-characterized mouse model of cardiotoxin (ctx)-induced muscle injury, we have demonstrated the involvement of the intrinsic pathway signaling in ctx-induced muscle cell death.
Objective: The objective of the present study was to characterize the role of c-Jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) for muscle cell death in ctx-induced injury and its mitigation by testosterone therapy.
Experimental Design: To investigate the contributions of JNK and p38 MAPK to muscle cell apoptosis, groups of 8 adult (8 wk old) male C57BL/6 mice received, under anesthesia, a single im injection of saline (control) or 100 µl of 100 µM ctx into the gastrocnemius muscles and were killed at 2, 4, 6, 8, 16, and 24 h after treatment. To examine the role of testosterone in preventing ctx-induced muscle cell apoptosis, groups of 8 adult male mice received a single sc injection of a long acting GnRH antagonist, acyline (20 mg/kg BW) to suppress endogenous testosterone production and empty, 1, or 2-cm testosterone-filled PDS capsules for 2 wks. Pretreated mice with GnRH antagonist and with or without testosterone supplementation received cardiotoxin in above-mentioned dose, and were killed 8 h later, the earliest time point with a marked increase in muscle cell death.
Results: Activation of JNK, as evidenced by a robust increase in phospho-c-Jun, was detected as early as within 2 h after ctx-treatment and remained active throughout the treatment. Ctx-treatment also led to activation of p38 MAPK at 4h, as evidenced by an increase in phospho-ATF-2, and remained active throughout the treatment period. Within the study paradigm, testosterone supplementation neither suppressed JNK and p38 MAPK activation nor prevented ctx-induced muscle cell death.
Conclusions: Collectively, the present study emphasizes the role of JNK/p38 MAPK-mediated intrinsic pathway signaling in ctx-induced muscle cell death. Testosterone therapy has no protective effect in acute muscle injury associated with increased muscle cell death after ctx-treatment.

Supported by a grant (GMO68510) from NIH.

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CALCITRIOL (1A,25-DIHYDROXYVITAMIN D_3) INHIBITS ANDROGEN GLUCURONIDATION IN PROSTATE CANCER LNCAP CELLS

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Molecular Endocrinology and Oncology, CHUL Research Center;
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Introduction: Androgens play a major role in the initiation and progression of prostate cancer, the second most common cause of cancer death in Americans. Derivatives of vitamin D such as 1a,25-dihydroxyvitamin D_3 (calcitriol) are considered as promising treatments for this type of cancer. Glucuronidation, catalyzed by UDP-glucuronosyltransferase (UGT) enzymes, is the most efficient inactivating pathway for androgens. In the human prostate, androgens glucuronidation is exclusively catalyzed by the UGT2B15 and UGT2B17 isoforms.
Objectives: This study was aimed at investigating the effects of calcitriol on the glucuronidation of androgens in prostate cancer cells.
Methods: Androgen-responsive prostate cancer LNCaP cells were treated with calcitriol. Glucuronidation activity was determined after incubation of androgens with cell homogenates. Calcitriol-induced changes in UGT mRNA and protein expression were examined by real time PCR and Western blot, respectively. The regulation of UGT expression was further elucidated by cotreatment experiments with calcitriol and the protein synthesis inhibitor cycloheximide or the androgen receptor (AR) antagonist casodex. Transient transfection of UGT promoter constructs and silencing RNA directed against AR were also performed.
Results: Calcitriol treatment of LNCaP cells drastically reduced the formation of androgen-glucuronides and inhibited UGT2B15 and UGT2B17 expression at both mRNA and protein levels. Experiments also revealed that the two UGT isoforms were differently regulated: calcitriol reduced UGT2B17 expression indirectly through activating successively the vitamin D receptor (VDR) and AR, while inhibition of the UGT2B15 gene transcription may occur through direct activation of VDR.
Conclusions: Results of the present *in vitro* study suggest that calcitriol treatment may increase androgen concentrations in prostate cancer cells. This effect is thought to limit the anti-proliferating actions of calcitriol. More effective vitamin D derivatives are currently tested for their differential effects on

prostate cancer and bone calcification. Based on our observations, we propose that these molecules should also be analyzed for their ability to modulate the UGT2B15- and UGT2B17-dependent glucuronidation of androgens in prostate cancer cells.

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LEPTIN AND GHRELIN BALANCE IS REQUIRED FOR MURINE SPERMATOGENESIS

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Reproduction is regulated by weight extremes. In that, hormones that regulate energy metabolism effect reproduction. Leptin, an adipose secreted hormone, is localized to the seminiferous tubules and its receptor is expressed by Leydig cells. Targeted deletion of leptin results in obesity and infertility. Ghrelin, first described as a regulator of energy balance, is the endogenous ligand of the growth hormone secretagogue receptor. This receptor (GHSR) is present in both Sertoli and Leydig cells, though its ligand is expressed only by mature Leydig cells. While mice lacking ghrelin are fertile, mice lacking leptin (*ob/ob*) are obese, infertile and exhibit impaired spermatogenesis. Thus, ghrelin and leptin are expressed in the testes without a known local function, but exhibit antagonistic actions on hypothalamic function. We propose that leptin and ghrelin act to regulate spermatogenesis locally in the testis, as well as centrally in the CNS. Accordingly, mice with a targeted disruption of ghrelin or its receptor (GHSR) were crossed to the leptin-deficient background (*ob/ob*). Wild-type, *ob/ob, ghrelin -/-, ghsr -/-* and *ob/ob//ghrelin -/- and ob/ob//ghsr -/-* mice were examined for testicular morphology, semen analysis and apoptosis by flow cytometry to discriminate between apoptotic, necrotic, and live cells. Testis sections from *ghrelin -/-* and *ghsr -/-* mice displayed slightly altered morphology. Compared to wild-type mice, Leydig cells appeared fewer in number and less well-developed, and some tubules displayed no germ cells. In contrast, seminiferous tubule lumens in *ob/ob* mice exhibited markedly reduced spermatogenesis and atrophied interstitial Leydig cells. Elimination of both ghrelin and leptin rescues the altered phenotype. Sections of *ob/ob//ghrelin -/- and ob/ob//ghsr -/-* testis revealed seminiferous tubules with abundant cellularity and mature sperm. Leydig and Sertoli cells displayed normal cell morphology. Flow cytometry or TUNEL analysis revealed that testes from *ob/ob* mice show reduced
apoptosis compared to wild-type mice. Levels of apoptosis were restored to wild-type levels when *ob/ob* mice were bred onto the *ghrelin -/- or ghsr -/-* backgrounds. Breeding *ghrelin -/-* or *ghsr -/-* mice onto the *ob/ob* strain rescues defective spermatogenesis and restores normal levels of apoptosis, suggesting that ghrelin leptin balance is essential for spermatogenesis.

Supported in part by T32DK007763 to DJL.

SPERMATOGENESIS/STEROIDOGENESIS/TESTIS BIOLOGY

16

EFFECT OF SUBCLINICAL AND OVERT HYPOTHYROIDISM ON TESTICULAR STEROIDOGENESIS

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Introduction and Objectives: The prevalence of goiter has been reported to vary from 2.3% to 68.8% in India. Hypothyroidism has been shown to be associated with a reduction in the testosterone level in males. The present investigation was undertaken to find out the step at which testicular steroidogenesis is inhibited and the functioning of testicular-gonadal axis, in hypothyroidism
Methods: Serum samples were selected from the subjects showing normal euthyroid, subclinical (TSH<10mIU/L) and overtly hypothyroid profiles. These samples were analyzed for the levels of LH, FSH, prolactin, testosterone, SHBG, progesterone and estradiol. Data was compared using student's t test.
Results and Conclusion: Our study shows that, subclinical and overt hypothyroidism both are associated with hypoandrogenemia, hyperprolactinaemia and a reduction in the circulating levels of progesterone and testosterone without any change in the level of estradiol, LH and FSH in comparison to the euthyroid controls. Bioavailable testosterone and SHBG both were decreased in overt hypothyroidism but not in subclinical hypothyroidism. Despite a fall in testosterone there is no change in FSH and LH indicating a defect in the steroidal biofeedback on the pituitary level. The lowering of testosterone could be due to inhibition of conversion of cholesterol to pregnenolone or pregnenolone to progesterone. Hyperprolactinaemia too could be a cause of hypoandrogenemia. T3 has primarily been shown to stimulate Leydig cell steroidogenesis. A fall in T3 in overtly hypothyroid patients could lead to an inhibition of testosterone production by the testis. However when T3 and T4 are within normal range, TSH could be considered to modulate basal steroidogenesis in testis. We have pooled data from euthyroid control and subclinical hypothyroid subjects where T3 and T4 levels are in normal range, and studied the correlation between the plasma levels of TSH, T3 and T4 levels with testosterone level. The testosterone level shows negative correlation with plasma TSH ($r=-0.359$, $p<0.05$) whereas T3 and T4 levels have no significant correlation with testosterone level. The results suggest that the TSH could be an important modulator of testicular steroidogenesis under normal condition. Its action may be masked by abnormal levels of T3 or T4.

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FUNCTIONAL STUDY OF HT31-8, A NOVEL HUMAN SPERMATOGENESIS-RELATED GENE

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The process of spermatogenesis is governed by expression of a series genes. Cloning of these genes and further functional study are very important to the elucidation of molecular mechanism involving in spermatogenesis. HT31-8 was cloned from human testis by differential cDNA microarray hybridization in our laboratory and predicted to edcode a PHD finger protein. Preliminary studies on mRNA level have predicted that HT31-8 is related to spermatogenesis.
Objectives: To investigate the role and mechanism of HT31-8 in the process of spermatogenesis.
Methods: Firstly, we examined the location of HT31-8 protein in adult testis by immunochemical analysis. Secondly, LZR-HT31-8 recombinant plasmid was transfected into mouse spermatogonia (GC-1 spg cell line), then the influence on the proliferation and apoptosis of germ cells were examined after the overexpression of HT31-8 by using flow cytometry, agarose gel electrophoresis and comet assay. Moreover, the pathway through which HT31-8 regulates cell proliferation or apoptosis was analyzed by screening differential genes using microarray hybridization. At last, the transcriptional regulating properties of HT31-8 were examined after HT31-8-Gal4 DNA-BD recombinant protein was transfected into CHO cells.
Results: 1) HT31-8 protein was expressed in the nucleus of spermatogonia, spermatocyte and spermatid, which implied that HT31-8 regulated biological process of spermatogenesis directly. 2) Cell cycles were compared and there was no remarkable difference between HT31-8 overexpression cells and control cells. After the inducement of Taxol (one of the agents which can induce apoptosis), percentage of apoptosis cells markedly increased in control group, whereas apoptosis percentage of overexpressed group was still low and had remarkably difference compared with control group. The resistance of HT31-8 against apoptosis was due to its positive effect on the expression of a series of anti-apoptotic genes and its negative effect on expression of some pro-apoptotic genes. These genes all acted on p53, Bcl-2 protein family and caspase. 3) HT31-8 can remarkably activate transcription from a reporter gene when fused to a heterologous DNA binding domain.
Conclusions: HT31-8 may be a novel transcript regulation gene in testis, and specifically involved in the resistance against revulsive apoptosis during spermatogenesis. Under the stimulus of some apoptotic signal, HT31-8 may activate the transcription of a series of anti-apoptotic genes, and restrain the transcription of some pro-apoptotic genes, which act on the **mitochondrial-**apoptosis pathway and resist apoptosis of germ cell.

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INFLUENCE OF ANDROGENS ON TESTICULAR CONNEXINS IN BROOK TROUT

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Gap junctions are composed of connexons which are themselves made up of transmembrane proteins termed connexins (Cxs). In mammals decreased Cx43 expression is associated with altered spermatogenesis. Brook trout are seasonal breeders and spermatogenesis is synchronous. As such these fish represent an excellent model in which to study the cellular processes implicated in spermatogenesis. We have previously shown in rainbow trout that there are 4 Cxs (Cx43, Cx43.4, Cx30, and Cx31) that are expressed in a stage and cell specific manner during spermatogenesis. To date, however, there is no information on the endocrine regulation of testicular Cxs in fish. The objective of this study was to determine whether or not androgens can directly regulate the
expression of Cxs in brook trout. Trout were sampled on a monthly basis from May to November during spermatogenesis. Histological examination of the testes indicated that fish were at Stage I in May and June; Stage II in July; Stage III in August; Stage IV in September-October and Stage V in October-November. Serum 11-KT levels were low (0.2 ng/ml) in June but increased progressively by 75-fold to peak levels in September. Levels decreased dramatically to less than 2.0 ng/ml afterwards. Levels of Cx43, which is expressed in Sertoli cells, and Cx30 which is expressed in Leydig cells, were elevated from June to September and significantly decreased by 60-80% in November at end of spermatogenesis. Levels of Cx43.4, which is expressed in spermatogonia and spermatocytes, increased from June to peak in July, progressively decreased by almost 75% in September and were undetectable thereafter. Levels of Cx31, which is expressed in endothelial cells, were constant from June until September and decreased by 80-90% thereafter. These results suggest that Cx levels vary according to stages of spermatogenesis independent of androgens. To determine if this was the case, in vitro assays using cultured seminiferous tubules were done. Tubules were cultured for up to 20 days in the presence of either 0, 1, 10 or 100 ng/ml of 11-KT. Tubules cultured with 0 or 1 ng/ml of 11-KT underwent spermatogenesis with most tubules reaching stages III and IV while the higher dose group reached stage V. Cx43 mRNA levels in the high dose group remained constant from days 5-15 but significantly decreased at day 20 when tubules reached stage V, suggesting that Cx43 levels were stage dependent and that these were not directly influenced by 11-KT. These data indicate that while high concentrations of 11-KT can influence spermatogenesis in the salmonid testis, it does not regulate Cx43 expression.

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EVIDENCE THAT ANDROGEN SUPPRESSION INDUCED STIMULATION OF SPERMATOGONIAL DIFFERENTIATION IN *jsd* MICE ACTS BY ELEVATING THE SCROTAL TEMPERATURE

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Male mice homozygous for the juvenile spermatogonial depletion (*jsd*) mutation undergo several waves of spermatogenesis; however, spermatogonial differentiation ceases and, in adults, spermatogonia are the only germ cells remaining. Either suppression of testosterone (T) or elevation of testicular temperature by cryptorchidism restored spermatogonial differentiation in these mice from a basal value of 4-8% in the untreated scrotal testis. When mice were cryptorchidized, it took only about a week to produce differentiated germ cells in 66% of the tubules and this increased to 96% in 2 weeks, with a simultaneous decrease in the testicular mRNA encoding the cold-inducible RNA-binding-motif protein 3 (*Rbm3*). In contrast hormone suppression alone stimulated differentiation in 6%, 32%, and 76% tubules, at weeks 1, 2 and 4, respectively, in the scrotal testis. Further, surprisingly, androgen ablation for four weeks dramatically reduced the hairless bare area on the scrotum and scrotal size, compared to the control mice. Intrascrotal temperatures were 34.1±0.4°C in the androgen-ablated *jsd* mice *vs.* 33.3±0.2°C in the controls, without changes in peritoneal temperatures. These results support the hypothesis that suppression of **T** is responsible for an increase in testicular temperature, which could be largely accountable for the stimulation of spermatogonial differentiation by hormone suppression. We presume that the anatomical changes in the scrotum occurred gradually over the 4 weeks of hormone suppression, explaining the more gradual stimulation of spermatogonial differentiation that occurs after suppression of T than after cryptorchidization. However, additional androgen ablation accelerated the differentiation process in cryptorchid testis, shown by the increased number of tubules with early spermatocytes (44% *vs.* 6%) at 1 week, indicating additional temperature-independent spermatogenic inhibitory action of T. (Supported by HD- 40397 from NIH).

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ROLE OF CASPASE 2 IN APOPTOTIC SIGNALING OF PRIMATE MALE GERM CELLS

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Objective: Caspase 2 is an initiator caspase whose activation has been found to promote apoptosis through mitochondria-dependent intrinsic pathway signaling in various cell systems, including during spontaneous male germ cell death. Previously, we have shown that the intrinsic pathway is the key pathway for male germ cell apoptosis in rodents, monkeys, and men. The present study investigates if germ cell apoptosis induced by mild testicular hyperthermia or deprivation of intratesticular testosterone (T) or after combined interventions involves activation of caspase 2.
Study Design: Groups of 8 adult Cynomolgus monkeys received one of the following treatments: 1) two empty silastic implants (C); 2) two 5.5 cm-**T** implants (T); 3) daily exposure of testes to heat (43C for 30 min) for 2 consecutive days (H); and 4) two T-implants plus exposure of the testes to heat for 2 consecutive days (H+T). Testicular biopsies were performed before and at 3, 8, and 28 days during treatment.
Results: Mean incidence of germ cell apoptosis increased significantly by d 3 in the H alone group and by d 8 in the T alone group but peaked at d 3 in H+**T** group. Maximum activation of caspase 2 in respective treatment groups, as evidenced by immunocytochemistry and immunoblotting using an active caspase 2 antibody, coincided with the increased incidence of apoptosis. In control testes, we detected moderate immunostaining for active caspase 2 in Sertoli cells with little or no expression of germ cells. In contrast, we found a strong staining for active caspase 2 in apoptotic germ cells and in the Sertoli cells. Co-staining for TUNEL and active caspase 2 further confirmed activation of caspase 2 only in those germ cells undergoing apoptosis.
Conclusion: Caspase 2 is activated in male germ cell apoptosis in nonhuman primates after heat stress, hormonal deprivation, or after combined interventions. Future studies aimed at determining the expression of inhibitor of apoptosis proteins in testis will be needed to determine why Sertoli cells are not dying in spite of enhanced expression of caspase 2.

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TESTICULAR HYPERTHERMIA BUT NOT HORMONE DEPRIVATION RESULTS IN STAGE-AND CELL-SPECIFIC ACTIVATION OF ERK AND INACTIVATION OF BCL-2

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In earlier studies, we have shown that the mitochondria-dependent (intrinsic) pathway is the key pathway for male germ cell apoptosis across species. To characterize the upstream signaling pathways that activate this death pathway, here we investigated the contributions ERK (extracellular signal-regulated kinase) and JNK (c-Jun NH2-terminal kinase) to male germ cell apoptosis in rats after hormone deprivation or heat stress. In the hormone deprivation model, rats were given a daily injection of vehicle for 14 days or GnRH-antagonist, acyline (1.6 mg/kg BW), for 2, 5, and 14 days. In the hyperthermia model, scrota of rats were exposed once to a temperature of 22C (control) or 43C for 15 min, and killed at ½, 2, and 6 h after heat treatment. Testicular hyperthermia but not hormone withdrawal led to sustained activation of ERK. Immunocytochemistry further revealed ERK activation only in the Sertoli cells involving exclusively heat-susceptible early (I-IV) and late (XII-XIV) stages within ½ h of heating. By 6 h, immunostaining for active ERK was, however, evident mostly in germ cells. Co-staining for TUNEL and phospho-ERK confirmed activation of ERK only in those germ cells undergoing apoptosis. Heat-induced germ cell apoptosis was also preceded by JNK activation, as evidenced by an increase in phospho c-Jun in testis lysates within ½ h of heating. Unlike ERK, phospho-c-Jun immunostaining was detected only in those heat-susceptible germ cells. Because activation of both ERK and JNK is known to induce BCL-2 phosphorylation leading to its inactivation, we next examined whether the increased germ cell apoptosis after heat stress is associated with BCL-2 phosphorylation. Compared with control, where no staining was detected, we found marked increase in serine-phosphorylated form of inactive BCL-2 only in heat-susceptible germ cells. Co-staining for TUNEL and phospho-BCL-2 confirmed phosphorylation of BCL-2 only in those germ cells undergoing apoptosis. The BCL-2 phosphorylation was not apparent during hormone deprivation-induced apoptosis. These results suggest that: 1) ERK and JNK promote male germ cell apoptosis via inactivation of BCL-2 through serine phosphorylation, and 2) ERK signaling is decisively different between these two paradigms and may represent an important step in the signal transduction pathway by which heat stress induces male germ cell apoptosis.

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ELEVATED SERUM INSL3 IS ASSOCIATED WITH FAILURE TO COMPLETELY SUPPRESS SPERMATOGENESIS IN MEN RECEIVING MALE HORMONAL CONTRACEPTION

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Background: The administration of testosterone plus a progestogen functions as a male contraceptive by inhibiting the release of the pituitary gonadotropins. After 3-4 months of treatment, most men are azoospermic or severely oligospermic (d"1 million sperm/ml). However, 10-20% of men have persistent sperm production despite profound gonadotropin suppression. Since insulin-like factor-3 (INSL3) has been shown to prevent germ cell apoptosis in mice, we hypothesized that INSL3 might be higher in men with persistent spermatogenesis during treatment with male hormonal contraceptives.
Methods: In a retrospective analysis, we measured serum INSL3 in 107 men from three recent male hormonal contraceptive studies and determined the relationship between suppression of spermatogenesis and serum INSL3.
Results: At the end-of-treatment sixty-three men (59%) were azoospermic and forty-four men (41%) had detectable sperm in their ejaculate. Baseline INSL3 did not predict azoospermia; however, end-of-treatment serum INSL3 was significantly higher in non-azoospermic men compared to those with azoospermia (129 ± 130 pg/ml vs. 86 ± 27 pg/ml; p=0.03). Furthermore, end-of-treatment serum INSL3 was positively correlated with sperm concentration (r=0.25; p=0.009) and was significantly associated with non-azoospermia by multivariate logistic regression modeling (p=0.03).
Conclusion: After six months of treatment with a hormonal male contraceptive regimen, serum INSL3 concentrations are higher in men with persistent spermatogenesis. Determining the exact role of INSL3 in maintaining spermatogenesis during gonadotropin suppression may provide insight into the failure of hormonal contraceptives to fully suppress spermatogenesis in some men. Furthermore, our data suggest that INSL3 could be a potential target for male contraceptive development.

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ROLE OF JNK IN TESTICULAR CELLS AFTER HYPERTHERMIA AND/OR INSTRATESTICULAR TESTOSTERONE DEPRIVATION TREATMENTS IN ADULT CYNOMOLGUS MONKEYS

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Objective: The JNK signaling pathway has been implicated in the activation of apoptosis in various cell systems. But its role in testicular germ cell death is unclear. The goal of this study was to define the role of JNK in male germ cell apoptosis in monkeys after mild testicular hyperthermia and/or deprivation of intratesticular Testosterone (T).
Study Design: Groups of adult Cynomolgus monkeys received one of the following treatments: 1) two empty implants; 2) two **T** implants (**T**); 3) daily exposure of testes to heat (H); and 4) two T-implants plus heat exposure (H+**T**). Testicular biopsies were performed before and at 3, 8, and 28 days during treatment.
Results: Activation of JNK was detected in all treatment groups. Compared to controls, a strong phospho-c-Jun staining was detected in the nuclei of apoptotic germ cells in all treatment groups and in the Sertoli cell nuclei in H and H+**T** groups. Co-staining for JNK 2, 3 and for **T**UNEL shows both isoforms only expressed in those apoptotic germ cells. In contrast, JNK1 was detected in the Sertoli cell nuclei at d 8 in H and H+T groups.
Conclusion: Our results indicate that: 1) JNK pathway may play a role in male germ cell apoptosis in monkeys; 2) JNK isoforms could have preferential effects on testis function; and 3) Sertoli cells participate in germ cell apoptosis triggered by heat stress via JNK signaling.

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PRE-TREATMENT OF SPERM WITH LOW HYPO-OSMOTIC SWELLING TESTS WITH CHYMOTRYPSIN PRIOR TO INTRAUTERINE INSEMINATION (IUI) AND AVOIDANCE OF UNPROTECTECTED INTERCOURSE RESULTS IN PREGNANCY RATES COMPARABLE TO IUI FOR OTHER MALE FACTOR PROBLEMS

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Males whose semen specimens show a hypo-osmotic swelling (HOS) test score <50% rarely achieve pregnancies with intercourse or intrauterine insemination (IUI). Conventional oocyte insemination with in vitro fertilization (IVF) allows normal fertilization rates but extremely low implantation rates.

Yet intracytoplasmic sperm injection (ICSI) allows normal pregnancy and implantation rates. These data suggest that the HOS abnormality may be related to a toxic factor that impairs the functional integrity of the sperm membrane, gets transferred to the zona pellucida by the attached sperm, which then becomes incorporated in the embryo membrane, which in turn causes a functional impairment that prevents embryo implantation. A previous study where sperm with low HOS scores were treated with the protein digestive enzyme chymotrypsin prior to IUI found only a modest improvement in pregnancy rates (3.3%) per cycle. We hypothesized that improvement may occur if the female partner is cautioned against unprotected intercourse to prevent the toxic untreated sperm from reaching the zona pellucida. The objective of this study was to reassess the efficacy of treatment of sperm with chymotrypsin for low HOS scores prior to IUI but with caution against unprotected intercourse prior to ovulation. IUI cycles using chymotrypsin treated sperm for HOS scores <50% were evaluated. Sperm with low motile density or morphology were still included in this group as long as the HOS score was <50%. Superovulation was not used. IUI was performed on natural cycles or with minimal gonadotropin stimulation to correct follicular maturation defects. IUI's were performed 40 hours after luteinizing hormone (LH) surge or human chorionic gonadotropin (hCG) injection. All cycles were supported by vaginal progesterone in the luteal phase. There were 155 matched pairs evaluated. The pregnancy rates per IUI cycle was 32.3% (n=50) for low HOS scores treated with chymotrypsin vs. 21.9% (n=34) for IUI's without chymotrypsin for male factor with normal HOS scores. The miscarriage rates were 34% (17/50) and 29% (10/34), respectively. Thus the live birth rate for low HOS scores was 21.2% (33/155) vs. 15.8% (24/155) for male factor with normal HOS scores per IUI cycle. Thus IUI may be tried before ICSI.

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ASSOCIATION OF SPERM DNA CYTOMETRY WITH ICSI OUTCOME

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Introduction and Objectives: Fertilization and subsequent development depends in part on the inherent integrity of the sperm DNA. Analysis of Sperm DNA content can be done by flow cytometry. It can classify sperm subpopulations into: condensed sperm chromatin (CSC), non-condensed sperm chromatin of haploid spermatid (non-CSC), diploid cell and sub-haploid sperm with low DNA content. Our objective was to investigate the DNA content of the sperm cells in infertile patients undergoing ICSI.
Methods: Semen samples were collected from 42 infertile men. Each sample was divided into 3 aliquots for routine semen analysis, for ICSI preparation, and for flow cytometry analysis. Sperm DNA was tested using the cell cycle/ DNA content assay by the flow cytometry. Patients were divided into two groups according to the pregnancy test: group I: positive pregnancy (n=17) and group 2: negative pregnancy (n=22).
Results: Table shows significant differences in CSC cells, non-CSC cells and sub-haploid sperm cells between the 2 groups ($p < 0.001$, <0.01, <0.001 respectively). Group 2 showed significant negative correlation between sub-haploid cells with CSC, non-CSC sperm cells and diploid cells ($r = -0.89, -.67, -0.39$). Similarly, pregnancy negative group also showed positive correlation between non-CSC with diploid cells. Group 1 showed significant negative correlation between haploid cells with non-CSC, and diploid cells ($r = -0.57, -0.59$).
Conclusion: Sperm DNA cytometry can be used to predict ICSI outcome. Sub-haploid cell level may be a predictor of ART outcome.

DNA content subpopulations	Group 1	Group 2
CSC	54.7 ± 3.9	18.3 ± 2.2 [a]
Non-CSC cells	5.8 ± 2.0	5.7 ± 1.0 [a]
Sub-haploid cells	28.2 ± 3.0	73.9 ± 2.8 [a]
Diploid cells	4.6 ± 2.2	1.9 ± 0.3
Results are expressed as mean ± standard error of mean; [a]significant difference between positive pregnancy test and negative pregnancy test groups.		

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EVALUATION OF MALE FACTOR AS A CAUSE OF SLOW CLEAVAGE BY EVALUATING INFERTILE DONOR/REICPIENT PAIRS HAVING FRESH EMBRYO TRANSFER (ET)

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Studies have demonstrated that embryos that cleave to only 4 or 5 blastomeres on day 3 are less likely to implant than 6-8 cell embryos. Theoretically embryos with slow cleavage could be related to either an egg or male factor. The objective of the present study was to determine by evaluating the outcome of oocytes fertilized by two different sperm whether the sperm considered abnormal by WHO criteria are more likely to result in embryos with a slower cleavage rate and thus a lower pregnancy rate (PR) and implantation rate. The source of the oocytes were from infertile donors. We were especially interested in infertile donors since they may have needed in vitro fertilization (IVF) because of a sperm problem. There were 151 paired ET cycles studies where a fresh ET was performed in both members of the pair. A sperm was considered abnormal if the sperm concentration was $<20x10^6$/mL, the motility <40%, or normal morphology using strict criteria <4%, hypo-osmotic swelling test <50%, or antisperm antibodies >80%. The donor-recipient pairs were divided into 4 groups: 1) both donors and recipients had all embryos with at least 6 blastomeres, 2) both had some embryos transferred with <6 cells, 3) donor no embryos <6 cells but recipient had one or more, 4) the donor had $\geq$1 embryo <6 but recipient all $\geq$6 cells. For group 3, where the donor had a mean blastomere number of 7.48$\pm$.96 and a viable PR of 58.3% (14/24) per transfer and an implantation rate of 36.5% (23/63), 62.5% (n=15) had male factor and yet the recipient group with a mean number of blastomeres of 5.89$\pm$.66 had only 25.0% (n=6) with male factor. The viable PR and implantation rates for the recipients was 45.8% and 22.5% (18/80) ($p<.<.07$ for implantation rate). For the other discordant pair (group 4), the percentage of infertile donor couples with male factor was 42.9% (9/21) and the mean blastomere number was 5.89$\pm$1.07 vs. only 14.3% (3/21) in the recipient group whose mean blastomere number was 7.38$\pm$.84. Group 4 did show significant differences in ongoing/delivered PRs 38.1% (8/21) for donors with low blastomere number vs. 71.4% (15/21) for recipients with good number ($p<.03$). Similarly implantation rates were higher in recipients – 22.0% (13/59) vs. 45.0% (27/60) with $p<.008$. These data do not show that a couple with a male with subnormal sperm according to WHO criteria is more likely to form embryos with slower cleavage. However the sperm could be responsible for slow cleavage but the defect is not associated with standard semen parameters in general.

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IDIOPATHIC MALE INFERTILITY IS RELATED WITH GAMETOGENESIS GENES EXPRESSION: RESULTS BY A FUNCTIONAL ANALYSIS OF GENE ONTOLOGY TERMS

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Introduction: Sperm analysis based in sperm count and motility has been employed for the diagnosis of male fertility for several decades. It is a useful tool to determine the fertile status of a male. There are still a significant number of infertile males where their sperm features, as determined by the basic sperm analysis are normal. Recent investigations have described the relevance of sperm mRNA in fertilization and early embryo development. Microarray technology informs about a wide range of mRNAs expression within a single experiment, and is ideal to analyze sperm expression profiles (SEP) in cells or tissues. Bioinformatic helps in the organization of such amount of results by following logical processes of gene expression grouping. Our aim with this work was to compare the SEP in spermatozoa obtained from males with idiopathic infertility versus those from sperm donors of proven fertility by employing microarray technology followed by a functional analysis, in order to determine the biological processes in male fertility that are different in infertile vs. fertile males.

Methods: Sperm samples were obtained from infertile males (n=5) presenting normal sperm count and motility (WHO criteria) parameters and donors (n=5) of proven fertility. Sperm mRNA was extracted using Trizol protocol, suspended in DEPC-treated water and frozen at -80 until the microarray experiments were performed in duplicate.

Results: Several groups of genes exhibit a significantly different proportion of the genes over or underexpressed in infertile males versus fertile sperm donors. In a different analysis level, different biochemical pathways or processes are implicated. Interestingly, when over or underexpressed genes in infertile males were grouped by their function, those involving spermatozoa differentiation were present at all analysis levels. Spermatid development, gametogenesis, spermatid differentiation, and male gamete generation processes present between 80-100% of their genes with a significant over expression in donors in comparison with infertile males showing normal sperm count. This suggests that infertility markers that may not be related with sperm production in terms of sperm count, but related with sperm function.

Conclusions: When the results of a differential microarray analysis between fertile and infertile males on spermatozoa are organized into functionally similar clusters, several biochemical or differentiation pathways are altered, but mainly those involved in male gamete formation. This is unequivocal evidence about the existence of several molecular infertility factors independent from the total sperm production. Microarray based sperm analysis is a promising diagnostic tool of male infertility, that needs to be further explored.

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ANIMAL MODEL STUDY OF A NEW PATIENT LUBRICANT'S AFFECT ON IN VITRO FERTILIZATION & EMBRYO DEVELOPMENT

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Experiments were done to evaluate in vitro fertilization and embryo development following sperm exposure to products used to lubricate devices in fertility medicine including: KY® Gel, Aquasonic® Ultrasound Gel and Pre'™ (a new Patient Lubricant recently cleared by FDA for use during fertility interventions). Bovine in vitro fertilization and embryo culture methods are standard and have been proposed as an excellent model for gamete toxicity studies (ReprodBioMed Online 2002;4:170-5). In this study, cryopreserved bull sperm (from a single bull) were routinely washed, resuspended in a TALP medium and placed into one of 5 treatments. These included: 1) Control sperm in medium alone; or sperm medium suspensions with the following added (v/v) 2) 10% Pre' lubricant; 3) 50% Pre' lubricant; 4) 10% KY®; or 5) 10% Aquasonic® Gel. Sperm were incubated in treatments for 30 min at body temperature, and placed into fertilization wells with mature oocytes (1 x 10^6 sperm cells per well). At 8 hrs, putative zygotes were transferred into embryo culture medium and further incubated. At 32 hr of culture, dividing embryos were counted (% fertilization in each treatment). Final development rates were evaluated on Day 7 (post IVF) to determine the % of total oocytes that had developed to the morula or blastocyst stage. ANOVA was used to compare the % fertilization of oocytes & the % of normal embryo development resulting from sperm in each treatment (as seen in Table below, data are mean +/- sd).

In Vitro Fertilization & Embryo Development After Sperm Exposure

Treatment	Total Oocyte Number	% Fertilized Oocytes (± sd)	% Embryos Developing (± sd)
Control Medium	80	61(5)[a]	40(9)[a]
Pre' 10%	80	60(8)[a]	39(8)[a]
Pre' 50%	80	59(6)[a]	43(10)[a]
KY 10%	80	23(6)[b]	6(5)[b]
Aquasonic10%	80	0[c]	0[c]

*[a,b,c] denote means that differ within column by p<0.0001(ANOVA).

Pre' Patient Lubricant did not interfere with the ability of sperm to fertilize oocytes or support embryo development in vitro (using a bovine model) even at high concentrations. Conversely, KY® and Aquasonic® significantly impacted the ability of sperm to fertilize oocytes, and allow normal embryo development.

Funded by INGfertility, the makers of Pre' Patient Lubricant & Pre~Seed Intimate Moisturizer.

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THE SIGNIFICANCE OF SPERM DNA OXIDATION IN EMBRYO DEVELOPMENT AND REPRODUCTIVE OUTCOME IN AN OOCYTE DONATION PROGRAM; A NEW MODEL TO STUDY A MALE INFERTILITY PROGNOSTIC FACTOR

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Background: Free efforts, there is still a great need for additional research on the applicability of free radical damage analysis in humans. One product resulting from free radical damage is the DNA-hydroxylation (DNA oxidation). Oocyte donation is a powerful tool to study sperm quality, since oocyte and endometrial characteristics can be controlled and are comparable among the recipients. To gain understanding about the effects of sperm DNA oxidation on embryo quality and reproductive outcome, we used pairs of oocyte donation cycles i.e. the same oocyte donors were employed and the only difference between the two treatments was the use of different sperm source.

Materials and Methods: Semen samples were obtained from couples undergoing oocyte donation cycles (n = 38). We obtained 76 semen aliquots before and after semen processing by swim up. A total of 19 oocyte donors were employed and they donated for two different recipients. Our model includes two original design features in order to overcome female factor heterogeneity: 1) sperm OXI DNA was determined in the same ejaculated sample that was used for ART and 2) ovum donation. We used the OXI DNA assay kit based on the direct binding of a fluorescent probe to the DNA adduct 8-oxoguanine in cells with damaged DNA and fluorescence was quantified using flow cytometry. We correlated sperm OXI DNA features with embryo quality parameters, and fertilization, implantation and pregnancy outcome. Furthermore, we grouped pairs of sperm samples with same oocyte donor, and calculated the difference in OXI DNA to correlate with the variation observed in the parameters of the embryos obtained from them.

Results: The relationship between the differences in the fertilization rates and IVF outcome parameters of the couples who shared the oocyte cohort (same donor) with

Conclusions: The association observed between early embryo quality and sperm DNA oxidation supports the relevance of the paternal contribution in the early stages of development (before the whole embryo translational machinery is activated). It highlights the importance of DNA oxidation defining sperm quality.

*p<0.05.	Fertilization (%)	Implantation (%)	blastomeres (n) (48 h)	Fragmentation (%) (72 h)	Triploid embryos
Fresh	-0.083	-0.131	0.317*	0.479*	0.288*
Swim-up	-0.406*	-0.220	-0.024	0.534*	0.206

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PATERNAL DNA DAMAGE SUPPRESSES *IN VITRO* PROLIFERATION OF MOUSE INNER CELL MASS

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Introduction ad Objectives: Sperm DNA damage is know to cause developmental failure and reduction in the numbers of live offspring and the effects of damages range as diverse as embryonic death and cancer susceptibility in the offspring. Here we report the *in vitro* proliferation ability of the inner cell mass of the embryos derived from the DNA damaged sperm and its association with *in vivo* implantation potential.
Methodology: Day 3.5 mouse embryos derived from the DNA damaged sperm were cultured on MEF feeder layer and proliferation ability of the inner cell mass was assessed for six days. *In vivo* implantation and post implantation developmental competence was studied by feto-placental analysis on day 18 of gestation.
Results: Development of embryos derived from 6 Gy irradiated sperm demonstrated heterogeneous growth on day 3.5 where approximately 1/3rd of the embryos failed to undergo compaction. The *in vitro* proliferation ability on feeder cells of sperm irradiated normally developed blastocyst on day 3.5 was indistinguishable from control embryos. In contrast, embryos showing delay on day 3.5 failed to form any outgrowth during 6 day of *in vitro* culture. The fetal number was reduced by 50% among sperm irradiated group although, the number of implantations were not affected.
Conclusions: The DNA damage in sperm can lead to preimplantation embryonic developmental delay resulting in defective ICM proliferation which could eventually cause post implantation embryonic death.

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LOWER SPERM DENSITY IS ASSOCIATED WITH HIGHER CALORIE AND FAT INTAKE

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Obesity is associated with decreased sperm density and total sperm count, and is a putative risk factor for male infertility. Since obesity reflects disturbed energy balance (intake>expenditure), mechanistic information could be derived from examining either of those components. We evaluated energy (caloric) intake and macronutrient composition of the diet in relation to sperm density, semen volume and total sperm count. Healthy, nonsmoking (n=60) and smoking (n= 89) men (20-51 y), with no history of reproductive problems and body mass index (BMI) ranging from 17.5-42.5 kg/m^2, provided a semen sample and completed a 106 item food frequency questionnaire (Block 95 FFQ). Low cutoff values for semen quality variables were: sperm density <20 X 10^6/mL, semen volume < 2.0 mL, and total sperm count < 40 X 10^6. Multivariable regression models were used to estimate the relative likelihood of having semen quality with increasing calorie and fat intake (% of calories), and to identify associations between BMI and semen quality. All models controlled for age, abstention, free plasma testosterone and smoking (cigarettes/day). Men with calorie intakes in the highest two tertiles were 3.2 times more likely to have low sperm density (95% CI: 1.1-9.3; $p<0.05$) and 5.2 times more likely to have low total sperm count (95% CI: 1.8-14.6; $p<0.01$) compared to men with calorie intakes in the lowest tertile. BMI was also inversely associated with low sperm density (â: -2.9 X 10^6 cells/mL; SE: 1.4; $p<0.05$). Calorie intake was significantly associated with reduced sperm density and total count in the subgroup of normal weight men, suggesting that calorie intake, rather than body fatness may explain associations between obesity and sperm quality. Controlling for calorie intake and the other covariates, men with fat intakes in the highest tertile were 2.9 times more likely to have low sperm density (95% CI: 1.2-7.3; $p<0.05$). Men with higher calorie and fat intakes were not more likely to have low semen volume, which may indicate testicular rather than secondary sex organ involvement. These results suggest that the link between obesity and reduced sperm density and total count can be at least partly explained by both the number and type of calories consumed. We postulate that hormones associated with eating (e.g. insulin, leptin, adiponectin) may be mediators of the reductions in sperm density and total count seen in obese men.

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CHARACTERIZATION OF THE PROTEOLYTIC ACTIVITY IN HUMAN SEMEN THAT REGULATES GALECTIN-3

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Galectin-3 is a carbohydrate binding protein that exhibits immunomodulatory and cell adhesion functions. Galectin-3 function is regulated, in part, by proteolytic cleavage that destroys the multivalency of the galectin-3 molecule, while preserving its carbohydrate-binding activity. Proteolytic enzymes implicated in the regulation of galectin-3 function include collagenases, leishmanolysin, elastases, matrix metalloprotease-13 (MMP-13), and the gelatinases MMP-2 and MMP-9. In human semen, galectin-3 was identified in spermatozoa, the soluble fraction of seminal plasma, and the crude membrane fraction of seminal plasma that includes prostasomes. Prostasomes are small membranous vesicles with immunosuppressive properties; thus, galectin-3 is implicated in the immunoregulatory functions of prostasomes. In the current study, the localization of galectin-3 to human prostasomes was confirmed by immunoblot analysis of prostasomes purified from seminal plasma by ultracentrifugation and size exclusion column chromatography. The intact galectin-3 protein (~ 30 kDa) and the cleaved galectin-3 CRD (carbohydrate recognition domain) variant (~16 kDa) were identified in prostasomes and in the soluble fraction of seminal plasma. A protease assay was developed to characterize the proteolytic activity in seminal plasma using biotinylated recombinant galectin-3 (brgal-3). The proteolytic activity that cleaves galectin-3 in seminal plasma was inhibited by the Zn^{2+} chelator 1,10 phenanthroline and enhanced

by Ca^{2+}/Mg^{2+} chelators EDTA and EGTA, suggesting that the protease is a MMP. To investigate the role of the gelatinases MMP-2 and MMP-9, which were previously identified in seminal plasma, gelatin-binding proteins were isolated by affinity chromatography and analyzed by silver staining and gelatin zymography. Protease assays demonstrated that gelatinases do not cleave galectin-3 in seminal plasma. Furthermore, a MMP-13 specific inhibitor failed to inhibit the proteolytic activity present in seminal plasma. These collective results suggest that MMP-2, -9, and -13 are not involved in the regulation of galectin-3 function by proteolytic cleavage in seminal plasma. In an attempt to identify the specific protease(s) that cleaves galectin-3 in seminal plasma, proteolytically cleaved brgal-3 was purified by avidin affinity chromatography. N-terminal sequencing of the cleavage site in brgal-3 by Edman degradation is in progress. These and future studies will provide insight into the role of galectin-3 in the immunomodulatory properties of seminal plasma and prostasomes in the female reproductive tract.

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SIGNIFICANT DECREASE IN SPERM DEOXYRIBONUCLEIC ACID FRAGMENTATION AFTER VARICOCELECTOMY.

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Introduction: Varicoceles are found in approximately 15% and 19-41% of the general and infertile populations, respectively, and have long been recognized as a common cause of infertility. The exact pathways of damage by varicocele are difficult to explain and may be due to apoptotic events or oxidative stress. Current research indicates that varicocele may be a causative factor for elevated sperm DNA fragmentation.
Objective: To measure sperm DNA integrity values before and after varicocelectomy.
Methods: This study is a retrospective analysis of eleven consecutive men with clinical varicocele and high levels of sperm DNA fragmentation as measured by the Sperm Chromatin Structure Assay (SCSA®). Each patient had a scrotal ultrasound to confirm the presence of either unilateral or bilateral varicocele and had a semen sample analyzed for level of DNA fragmentation using the SCSA. All patients had a DNA Fragmentation Index (DFI) over 27-30% (fair to poor sperm DNA integrity) and had no other potential obvious reasons for high levels of sperm DNA fragmentation and infertility other than the presence of a varicocele(s).
Results: Ninety-percent of the patients showed a significant decrease in sperm DNA fragmentation levels.
Conclusions: Although this study was small, ten of the eleven varicocele patients showed a significant decrease in sperm DNA fragmentation after varicocele repair. Elevated sperm DNA fragmentation has been shown to have a significant negative effect on pregnancy outcome using *in vivo*, IUI, routine IVF and to a lesser extent ICSI fertilization; therefore pregnancy outcome may improve after varicocelectomy.

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CONCEIVING A CHILD AFTER VASECTOMY

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Vasectomy is a permanent and perfect male contraception procedure. This procedure is regretted in about 5% of all cases. This percentage seems to increase. Vasovasostomy (VVS) is the first choice of treatment to fulfil the desire to have a child. If after VVS within a year no pregnancy is realized, assisted reproductive techniques (ART) will be employed. Possibly in combination with PESA or TESE. In literature pregnancy-and patency rates are frequently used as primary outcome of treatment. Therefore this study evaluates the real number of births as a primary outcome.
From 1991 to 2004, 190 patients from both centres were analysed. A single urologist operated all men performing a microsurgical 2-layer anastomosis described by Silber. Data on childbirth after VVS and ART were obtained from medical records and by contacting patients by telephone. Data were completed for 162 out of 190 patients. In total 146 men were analysed, whereas 16 dropped out of this study because their desire to have a child was not maintained after VVS. The population had a mean obstruction-interval of 9,3 years (+/- 4,8) and 31 (22%) of them had azoospermia after VVS. Partners' mean age was 32,0 years (+/- 4,5). VVS was only completed unilaterally in 19 out of 146 men, 19 VVS were a redo. In 36 relationships 50 children were born after VVS. In the remaining 110 men only 82 decided to make use of ART. In this group 64/82 used either IUI or IVF/ICSI, 14/82 underwent PESA with IVF/ICSI, and 4/82 underwent TESE with IVF/ICSI. This resulted in another 62 children out of 47 relationships. During the course of ART, 3 spontaneous pregnancies occurred, which were therefore excluded from this group. From a group of 146 men, 115 made use of all currently available techniques (VVS with or without ART) that were advised to them. This resulted in 112 children from 83 men.
In conclusion we present the long-term paternity outcome of a group of 146 vasectomized men, with prognostic unfavourable parameters for VVS. In men that utilized all current available medical techniques their wish to conceive a child was fulfilled in 72,2% (83/115). When men regret their vasectomy they like to know their real chance to conceive a child. Because of the possibility that VVS is not successful thorough counselling of patients before this operation should include ART and the use of surgically retrieved spermatozoa.

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CO-LOCALIZATION OF SINGLE-STRANDED DNA AND HISTONE H2B IN HUMAN SPERM NUCLEI: INSIGHT ON THE ETIOLOGY OF SPERM DNA DAMAGE

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Numerous studies have shown that infertile men possess high levels of sperm DNA damage. However, little is known about the etiology of this damage. The objective of this study was to localize the foci of single-stranded (ss) DNA and histone H2B in human sperm nuclei. Smears were prepared from semen samples of fertile and infertile men. We examined the specific localization of sperm nuclear ssDNA and histone H2B by immunocytochemistry and evaluated the co-localization of H2B and ssDNA immunostaining. The ssDNA and histone H2B immunocytochemistry studies demonstrated one of two sperm nuclear staining patterns: 1) faint, punctated staining and 2) intense and diffuse nuclear staining. The majority of spermatozoa (60%) exhibited faint, punctated staining for both H2B and ssDNA. Spermatozoa exhibiting diffuse H2B nuclear staining (representing 20% of all spermatozoa) consistently demonstrated diffuse ssDNA nuclear staining with specific co-localization of H2B and ssDNA staining. In contrast, of the spermatozoa exhibiting diffuse ssDNA nuclear staining (representing 39% of all spermatozoa) only 41% demonstrated diffuse H2B nuclear staining with specific co-localization of H2B and ssDNA staining. Inter-observer variability in the assessment of H2B and ssDNA co-localization was 15%. Positive (DNAse-treated nuclei) and negative controls (S1 nuclease-treated nuclei and absence of primary antibody) were obtained to validate the specificity of the ssDNA antibody. Western immunoblots were used to verify the specificity of the H2B antibody. Our data show that human sperm nuclei generally exhibit faint and discrete (presumably peripheral) foci of ssDNA and histone H2B. The co-localization studies (1) indicate that abnormal sperm nuclear histone (H2B) retention is consistently and specifically associated with DNA damage (ssDNA) and (2) suggest that in a population of ejaculated spermatozoa, the etiology of sperm DNA damage (ssDNA) is multi-factorial, with nearly 50% of the damaged cells exhibiting histone retention.

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TEMPERATURE-DEPENDENT COLD SHOCK DAMAGE TO PORCINE SPERM CORRELATES WITH PROTEIN TYROSINE PHOSPHORYLATION.*

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Porcine sperm are extremely sensitive to the damaging effects of cold shock. For this reason, most boar semen used for artificial insemination is stored and shipped at 15-17°C. It is not unusual for shipped semen to become accidentally cooled below this temperature, particularly during the cooler months of the year, resulting in cold shock damage to a proportion of the sperm and decreased fertility of the sample. A method to evaluate the degree of cold shock damage in shipped boar semen would be of great benefit to swine producers. Our previous study demonstrated an increase in protein tyrosine phosphorylation (PY) following cold shock. The objective of this study was to further characterize the effects of cold shock on porcine sperm viability, spontaneous acrosomal exocytosis (sAR), and protein PY. A total of 7 experiments using individual semen samples from 4 adult boars were performed. Sperm were suspended in capacitation medium containing 0.04% BSA, equilibrated for 15 min at 30°C, and then transferred to waterbaths set at 0-18°C in 2°C increments for 10 min each. Samples were removed before and after cold shock for evaluation of sperm viability (carboxyfluorescein diacetate/ propidium iodide fluorescence), sAR (Coomassie G-250 staining of paraformaldehyde-fixed samples), and antiphosphotyrosine immunoblotting of extracted sperm proteins followed by densimetry via image analysis. We report here that porcine sperm display temperature-dependent cold shock damage that directly correlates with protein tyrosine phosphorylation, as assessed by Pearson Product Moment Correlation (correlation coefficients provided in parentheses below, all P values <0.001). The percentage of dead sperm was inversely correlated with temperature (-0.921) with some individual boar variation in lethal temperature, but similarly shaped cold shock curves. Protein PY was directly correlated with sAR (0.965). Both sAR and protein PY correlated with percentage of dead sperm (0.903, 0.922), and inversely correlated with temperature (-0.910, -0.915). These results indicate that protein tyrosine phosphorylation is a sensitive and quantifiable marker of porcine sperm cold shock damage. Future studies will focus on the development of a commercial protein PY assay of porcine sperm cold shock damage for use by swine producers.

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COMPUTER ASSISTED SPERM HEAD MORPHOLOGY ASSESSMENT AND ITS CORRELATION WITH SPERM DNA DAMAGE

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Introduction and Objectives: Sperm chromatin structure of poor quality may be indicative of male subfertility. Evaluation of sperm chromatin structure is an independent measure of sperm quality that provides good diagnostic and prognostic capabilities. Therefore, it may be considered a reliable predictor

of couple's inability to become pregnant. Computer-assisted sperm morphometry analysis has improved the assessment of sperm morphology which may be associated with sperm DNA fragmentation. Our objective was to evaluate the correlation between computerized analyses of sperm head morphology and DNA fragmentation.
Methods: A total of 21 semen samples of infertile patients were tested. Smears were stained by Diff-Quik and sperm head morphology was analyzed by Sperm Morphology Analyzer (Sperm class analyzer software, Microptics, Barcelona, Spain) according to the manufacturer's instructions. Sperm DNA damage was analyzed by Comet assay (single cell gel electrophoresis). Morphology was divided according to head size (normal, macro, micro, pin head and phantom head) and head shape (normal, round, thin, paintbrush, narrow base and amorphous head). Sperm DNA fragmentation was classified into mild, moderate and severe according to the tail moment.
Result: Both micro and amorphous head were positively correlated with severe DNA damage ($r = 0.48$ and 0.48 respectively, $P < 0.001$ and $P < 0.05$) and negatively correlated with mild DNA damage ($r = -0.58$ and -0.49, $P < 0.001$ and $P < 0.001$ respectively). No significant correlation was found between DNA damage and other head abnormalities.
Conclusions: Sperm with amorphous and micro head abnormalities have higher DNA fragmentations compared to other forms of sperm head abnormalities. Amorphous and micro head abnormalities are lower in patients with mild DNA damage. Computerized analysis of sperm head shape and size may be indicative of extent of DNA damage.

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SIGNALING & SIGNALING RECEPTOR TRANSCRIPTS: DISRUPTION IN SEVERE HUMAN TERATOZOOSPERMIA
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Introduction: Neuropeptide and neurotransmitter families of signaling proteins and receptors are required for both synaptic transmission and the successful genesis of viable mammalian spermatozoa. The signaling
appears disrupted in male teratozoospermia such that even when motile spermatozoa contact the oocyte they may fail to initiate the acrosome reaction. In such cases ICSI is then required for successful fertilization.
Objectives: The testable hypothesis was: teratozoospermic individuals will present disruption of signaling receptor spermotozoal RNAs. This may be informative to understanding one aspect of this phenotype. The hypothesis was challenged by examining the distribution of spermotozoal RNAs present in teratozoospermia.
Methods: A microarray study employing Affymetrix U133 (version 2) arrays was undertaken. This compared the transcripts carried in spermatozoa from 13 fertile males to those from 8 individuals diagnosed as severely and consistently teratozoospermic. The significance level for subsequent analysis was assessed relative to a random sample-class permutation.
Results: Transcripts for a large number of proteins whose primary association has been cell-cell neuronal signaling were detected. The transcriptional abundance of mRNAs for specific GABA and glutamate receptor proteins linked to the initiation of the acrosome reaction was disrupted. Promoter analysis to identify transcription factors common to the transcripts suggested that the BRN pathway may be involved in the disruption.
Conclusions: Spermatozoal RNAs likely provide a fruitful and non-invasive proxy to examine the genetic origins of male factor infertility. Here we suggest that failure exhibited by teratozoospermia to undergo the acrosome reaction may reflect the disruption of several transcription factor pathways including the BRN pathway.
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NORMAL SPERM MOTILITY REQUIRES THE PRESENCE OF JUNCTIONAL ADHESION MOLECULE-A (JAM-A)
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Introduction: Junctional adhesion molecule-A (JAM-A) is a cell surface protein, which belongs to the immunoglobulin superfamily whose members are characterized by having two extracellular Ig domains which mediate heterophilic and homophilic interactions. In general, JAM proteins (A, B, C, D, and L) play multifunctional roles in a variety of cellular processes and are involved in cell-cell adhesion and the assembly of tight junctions in epithelial and endothelial cells. Recently JAM-C, which shares 33% identity with JAM-A, was shown to be critically essential for sperm development in mice via its role in the assembly of the cell polarity complex in round spermatids. While studying the role of JAM-A *in vivo*, we observed that JAM-A knockout (K/O) mice have significantly ($P<0.05$) smaller mean litter sizes.
Objective: Our goal was to determine if the male gamete plays a role in the subfertility associated with JAM-A deficiency, by performing expression analyses in the testis, as well as functional studies on sperm.
Methods: Developmental RT-PCR was performed on the testis, and protein expression was determined in both testis and sperm using several techniques. Finally, sperm motility was analyzed using computer-assisted analysis (CASA).
Results Obtained: In wild-type (WT) mice transcripts were detected in the testis as early as 4 days post-parturition and throughout adulthood, where histological preparations showed the protein to be localized at the Sertoli-Sertoli cell tight junctions. In WT, but not K/O, mice Western analysis showed the presence of the 32 kDa protein in testis and sperm. This was confirmed by flow cytometry and immunocytochemistry where JAM-A has a punctate appearance on the plasma membrane overlying the acrosome and midpiece of the flagellum, the site of the mitochondrial sheath. CASA revealed highly significantly reduced motility ($P<0.001$), progressive motility ($P<0.001$), and hyperactivity ($P<0.01$) for JAM-A K/O sperm compared to the WT. These defects were seen both before and after capacitation, with the latter being more severe.

Conclusion: Our studies show that JAM-A is required for normal sperm motility. To elucidate the mechanism of action of JAM-A in murine sperm motility, studies are underway to use immunoelectron microscopy to sub-localize JAM-A within microdomains of the flagellum, and to determine its possible involvement in signal transduction.

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A RECOMBINANT SEMINAL PROTEIN COMBATS INHIBITORY EFFECTS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETs)

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Neutrophils ordinarily infiltrate the female reproductive tract subsequent to mating or artificial insemination, resulting in reduced fertility. Recently, it was demonstrated that equine neutrophil extracellular traps (NETs) ensnared sperm, interfering with their normal transport through the female reproductive tract. A major constituent of NETs was found to be DNA extruded from non-apoptotic neutrophils. Seminal plasma (SP) or proteinaceous extracts from SP inhibited sperm-neutrophil binding, and specifically degraded sperm-activated NETs, without suppressing bactericidal activity of neutrophils. Fertility associated antigen (FAA) is a 31 kDa protein produced in the accessory sex glands which binds to sperm as they traverse the male urogenital tract. FAA shares 87% identity with DNase-I-like family members and contains two internal peptide sequences with conserved DNase-I signature motifs. The purpose of this study was to determine if FAA displayed DNase activity and inhibited sperm-neutrophil binding. To that end, a recombinant bovine FAA (rbFAA) construct spanning 603 bp, including both DNase-I motifs, was cloned from seminal vesicles. The construct, designed to contain a C-terminal $(His)_{6x}$ tag in a pCR T7/CT-TOPO expression vector, was expressed in E. coli. The expressed recombinant (231 aa, 26.6 kDa) was purified by metal affinity chromatography to near homogeneity. Purified rbFAA displayed DNase activity at concentrations of ≥3?M as visualized by agarose gel electrophoresis. Sperm from 9 bulls were incubated with various concentrations of rbFAA or SP protein extracts, washed 3X, and sperm were mixed with freshly isolated bovine neutrophils (15×10^6 cells/ml). After 30 min., the proportion of sperm-neutrophil binding was observed microscopically. Whole SP protein extracts at concentrations of 200 or 400 ?g/ml inhibited binding by 28 and 40 %, respectively ($P<0.01$), compared to control. Concentrations of 80 or 160 ?g/ml rFAA displayed similar activity to whole seminal plasma extracts, reducing binding by 38 and 44 %, respectively ($P<0.001$), compared to control. When diluted SP from 6 bulls was incubated with plasmid DNA, degradation indicated that seminal DNase activity varied widely across males. The ability of FAA to inhibit sperm-neutrophil binding provides a potential mechanism to explain the increased fertility observed with FAA-positive bulls. In pigs and horses, co-insemination of extended semen with SP significantly improved pregnancy rates, and litter size also increased in pigs. Whether or not that effect is the result of seminal DNase activity, contributed by FAA, remains to be determined experimentally.

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IDENTIFICATION OF SRC TYROSINE KINASE IN HUMAN SPERMATOZOA AND ITS ROLE DURING CAPACITATION

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Tyrosine phosphorylation of proteins is one of the main processes associated with the development of some specific functions of ejaculated human spermatozoa. Although this process as well as the identity of the phosphorylated targets have been well characterized, only few tyrosine kinases (TKs) have been identified so far. Moreover, their roles in regulating sperm functions as well as their relation with serine-threonine kinases, such as protein kinase A (PKA), are still unknown.
In the present work, we report the presence and localization of Src kinase in ejaculated human spermatozoa and investigate the role played by this TK during capacitation and its link to PKA activity. Immunoprecipitation and western blot analysis of protein lysates from human spermatozoa using specific anti-p60src antibodies identified a single band of about 70 kDa molecular weight. Immunofluorescence analysis of fixed and permeabilized sperm localized positivity mainly in the post-acrosomal region of sperm head and midpiece in over 80% of the sperm population. By both immunoprecipitation and immunofluorecence techniques with antibodies recognizing tyrosine phosphorylation of Src at 416 (pY416) or at 527 (pY527) positions, which identify the active or inactive kinase respectively, we were able to demonstrate an increased phosphorylation in Y416 during sperm capacitation achieved in vitro. Blocking Src activity by addition of its inhibitor SU6656 either during or at the end of capacitation resulted in a significant reduction in tyrosine phosphorylation of sperm proteins, in particular in the 80-115 kDa molecular weight range, without affecting phosphorylation of protein kinase A anchoring protein, AKAP3. Moreover, such inhibitor completely blocked progesterone-induced acrosome reaction and interfered with calcium response to progesterone evaluated in fura-2 loaded sperm, reducing in particular the peak phase. No effects on sperm motility and hyperactivation parameters evaluated by computer assisted sperm analysis (CASA) resulted from incubation of sperm with SU6656, independently on the time of addition to sperm.. Finally, by the use of TK and PKA inhibitors (erbstatin A and H89, respectively), we demonstrated that Src activation during capacitation is dependent on tyrosine kinase but not on PKA activity.
All together these findings suggest a pivotal role exerted by a novel isoform of Src in human sperm capacitation and acrosome reaction.

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THE HEALTHY MEN STUDY: AN EVALUATION OF EXPOSURE TO WATER DISINFECTION BY-PRODUCTS AND SPERM QUALITY

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Introduction and Objectives: This study examined if continued or intermittent exposure of men to disinfection by-products (DBPs) at levels found in public drinking water, may produce testicular toxicity as evidenced by altered semen quality. Chlorination of tap water generates DBPs, which have demonstrated reproductive toxicity in rodents at high doses. One class of DBPs, the haloacetic acids (HAAs), has been shown to disrupt spermatogenesis in rats, suggesting that HAAs in tap water could pose a reproductive risk in men.
Methods: We conducted a prospective cohort study to evaluate semen quality in men with well-characterized exposure to DBPs varying from minimal to near the maximum concentration limits set by the US EPA. Participants were 228 presumed fertile male partners of women enrolled in a cohort study on DBPs and early pregnancy loss from 3 geographic study sites with different DBP profiles. They completed a telephone interview about demographics, health history, water consumption and other exposures and provided a semen sample. Semen outcomes included semen volume, sperm concentration, and sperm morphology, as well as DNA integrity as measured by the sperm chromatin structure assay and chromatin maturity determined by chromomycin A3 staining. Exposures to DBPs were evaluated as: (1) the DBP concentration (ìg/L) in water, (2) the product of the DBP concentration in water and volume of water ingested by each participant (ìg/Day), and (3) the concentration of DBP in water and time spent bathing and showering (ìg/Day). We used multivariable linear regression to assess the relationship between exposure to DBPs and adverse sperm outcomes, adjusting for age, days abstaining, and education.
Results: The mean (median) sperm concentration (million/mL) and sperm count (million) were 114.2 (90.5) and 389.8 (276.7), respectively. These sperm parameters were not associated with exposure to DBPs. No consistent pattern of an increase in abnormal semen quality with elevated exposure to DBPs was found for other semen quality parameters. Neither the use of alternate methods for assessing exposure to DBPs nor site-specific analyses materially changed these results.
Conclusions: The results of this study do not support an association between low-level exposure to DBPs and adverse sperm outcomes. *Disclaimer: This abstract does not reflect EPA policy.*

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ANALYSIS OF THE ROLE OF INPP5B IN SPERM FUNCTION AND MALE INFERTILITY

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Mice deficient in the type-2 inositol polyphosphate 5-phosphatase Inpp5b have defects in male fertility. We are studying this knockout mouse as a model to understand the contributions of (a) sperm maturation in the epididymis and (b) synthesis and processing of ADAM (A Disintegrin and A Metalloprotease) proteins on the sperm to male fertility and sperm function. Sperm from *Inpp5b*$^{-/-}$ mice have reduced motility and ability to fertilize eggs *in vitro* (Dev Biol.240:641). Following up on this study, we found that poor motility is not the sole cause of reduced fertility; if motile sperm from *Inpp5b*$^{-/-}$ mice are selected by a swim-up preparation and tested for their ability to fertilize ZP-free eggs, these sperm still do not fertilize eggs *in vitro* to the same extent as controls. We then tested whether the failure to fertilize was a result of reduced binding, reduced fusion, or failure to activate eggs. IVF binding assays showed that sperm from *Inpp5b*$^{-/-}$ mice are deficient in binding to the egg plasma membrane and subsequent fusion with the egg when compared to both wild-type and heterozygous controls, but the *Inpp5b*$^{-/-}$ sperm are capable of activating eggs if they do fuse with the egg. Analysis of binding and fusion data from the IVF studies used a two-way nested ANOVA, a valuable tool that can take into account the day-to-day IVF variability and variability among males regardless of genotype. A second abnormality observed in sperm from *Inpp5b*$^{-/-}$ mice is reduced proteolytic processing of the sperm protein ADAM2 (Dev Biol.240:641). This processing normally occurs during transit of sperm through the epididymis. We show here that processing of ADAM3 also is defective in *Inpp5b*$^{-/-}$ sperm, and that the severity of the deficiency in ADAM processing is variable from male to male. There are two possible explanations for the abnormal ADAM cleavage: (a) reduced cleavage of ADAMs on the sperm surface as sperm go through the epididymis and/or (b) reduced transit of ADAMs to the surface during protein biosynthesis in developing sperm. Inpp5b is expressed in epididymal epithelial cells and at low levels in spermatocytes and spermatids. Previous work has shown that a conditional knockout of *Inpp5b* specifically in spermatids did not cause the fertility defects seen in the *Inpp5b*$^{-/-}$ mice. Since Inpp5b is expressed in the epididymis and there is a defect in ADAM cleavage in *Inpp5b*$^{-/-}$ mice, defects in epididymal maturation of sperm could be the major cause of the infertility in *Inpp5b*$^{-/-}$ mice. We are also currently investigating if sperm from *Inpp5b*$^{-/-}$ mice have a reduction in surface ADAMs, a phenotype that has been observed in a number of knockouts with abnormalities in sperm function.

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OUABAIN STIMULATES PROTEIN PHOSPHORYLATION IN RAT SPERMATOZOA VIA THE NA,K-ATPASE

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The hormone ouabain through its receptor, the Na,K-ATPase triggers an intracellular cascade of protein phosphorylation events in several cell types via the epidermal growth factor receptor (EGFR) and the MAP kinase pathway. The objective of the present work was to determine whether ouabain has this effect in rat epididymal spermatozoa. Overnight incubation with nanomolar concentrations of ouabain stimulated tyrosine kinase protein phosphorylation in the gametes, as identified by immunoblotting with anti-phospho-tyrosine antibodies. The pattern of protein phosphorylation was similar to that caused by cAmp in the cells, and could be observed not only under non-capacitating conditions, but to a lesser extent in media that supported sperm capacitation. AG1478, and inhibitor of EGFR and UO126, an inhibitor of the mitogen activated protein kinase (MEK) blocked ouabain induced protein phosphorylation in the cells. The protein kinase inhibitor H89 caused a similar effect. Two Na,K-ATPases composed of the alpha1 and alpha4 catalytic subunits are expressed in rat spermatozoa. At present, the ability of each alpha isoform in mediating ouabain phosphorylation signaling is unknown. This was explored after separate expression of the Na,K-ATPase alpha polypeptides in insect cells using baculoviruses. Both alpha1 and alpha4 isoforms were able to stimulate protein phosphorylation in the host cells. However, as expected by the differences in ouabain affinity reported for the isoforms, protein phosphorylation in cells expressing alpha1 required higher concentrations of ouabain than those expressing alpha4. Altogether these results suggest that ouabain is a factor that acting through the Na,K-ATPase alpha isoforms can trigger a cascade of phosphorylating events in rat spermatozoa.

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BETA-CYCLODEXTRIN PLUS CHOLESTEROL REVERSIBLY INHIBITS PORCINE SPERM CAPACITATION*

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Previous studies suggest that cholesterol efflux may not be necessary for in vitro porcine sperm capacitation. Conversely, we have shown that the incubation of porcine sperm with 2-hydroxypropyl-beta-cyclodextrin plus cholesterol 3-sulfate (CD/ChS), a treatment assumed to favor cholesterol uptake, inhibits porcine sperm capacitation. Treatment with CD/ChS also protects porcine sperm from cryodamage. However, for this treatment to become applicable, it is necessary to demonstrate that sperm capacitation is not permanently inhibited. This is the objective of our study. A total of 5 experiments using different semen samples from 4 adult boars were performed. Sperm were incubated for 3h in capacitation medium containing 0.04% BSA, 0.8 mM CD and 0.5 mM ChS (CD/ChS) or 0.8 mM HBCD alone (CD). Following the 3h incubation, duplicate CD/ChS samples were diluted 1:5 with either CD/ChS or CD medium and incubated for an additional 3h. Samples were removed at 3h and 6h for evaluation of sperm viability (carboxyfluorescein diacetate/ propidium iodide fluorescence), calcium ionophore-induced acrosome reaction (iAR) (Coomassie G-250 staining of paraformaldehyde-fixed samples, data represent differences between induced and uninduced samples), and antiphosphotyrosine immunoblotting of extracted sperm proteins followed by densimetry via image analysis (protein PY). Protein PY correlates with porcine sperm capacitation under standard conditions and is expressed as a percentage of positive control (3h incubation with cAMP agonists). Data given as means $\pm$ SEM and analyzed by 2-way ANOVA (treatment and boar) followed by Tukey's multiple comparison test. As previously reported, 3h incubation with CD/ChS inhibits sperm capacitation as assessed by iAR (7.0 $\pm$ 1.9%) and protein PY (6.5 $\pm$ 2.0%). In contrast, sperm capacitated for 3h with CD have significantly increased capacitation as assessed by by iAR (43.0 $\pm$ 7.0%) and protein PY (57.8 $\pm$ 7.8%). Sperm that were incubated for 3h with CD/ChS and then an additional 3h following 1:5 dilution in CD/ChS still displayed inhibited capacitation at 6h (iAR: 1.4 $\pm$ 5.9%; protein PY: 9.7 $\pm$ 2.9%). In contrast, incubation for 3h with CD/ChS followed by 1:5 dilution with CD resulted in sperm capacitation at 6h (iAR: 46.9 $\pm$ 7.8%; protein PY: 49.6 $\pm$ 7.1). These results demonstrate that the inhibitory effect of CD/ChS on in vitro porcine sperm capacitation is reversible. Moreover, these data suggest that porcine sperm capacitation status is directly related to cholesterol content.

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FERTILIZATION/GERM CELL DEVELOPMENT/REPRODUCTIVE DEVELOPMENT

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MULTIPLE STEPS IN THE FERTILIZATION CASCADE ARE IMPAIRED WHEN SPERM GLYCOLYSIS IS DISRUPTED

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Glyceraldehyde 3-phosphate dehydrogenase-S (GAPDHS) is a sperm-specific glycolytic enzyme in mammals. Male mice lacking this enzyme are infertile and produce sperm with very low ATP levels and severe motility defects (Miki *et al.*, 2004; PNAS 101:16501). We examined multiple steps in the fertilization cascade to further assess the functional competence of sperm lacking this enzyme. GAPDHS-null sperm did not exhibit tyrosine phosphorylation when

incubated in capacitation medium for 2 h. The addition of a potent, cell-permeable cAMP analog (0.1 mM cBiMPS) and a phosphodiesterase inhibitor (0.1 mM IBMX) induced tyrosine phosphorylation in wild-type sperm, but not in GAPDHS-null sperm. These results indicate that glycolysis is required for capacitation-dependent tyrosine phosphorylation, even under conditions that elevate intracellular cAMP levels. GAPDHS-null sperm also have significantly reduced zona binding capacity. In parallel binding assays we typically observed less than 10 GAPDS-null sperm/oocyte compared to more than 25 wild-type sperm/oocyte. In contrast, no defects in induction of the acrosome reaction were detected. Similar percentages of wild-type and GAPDHS-null sperm were acrosome reacted following incubation with calcium ionophore (53-57%) or heat-solubilized zonae pellucidae (46-47%). Compared to *in vitro* fertilization (IVF) rates >50% with sperm from wild-type males, GAPDHS-null sperm rarely achieved IVF and oocytes that appeared to be fertilized never developed beyond the 2-cell stage. We also compared IVF rates after zona drilling using a laser ablator, which increased the fertilization rate with GAPDHS-null sperm to 30.3%. In these experiments, development to the blastocyst stage was indistinguishable following fertilization with GAPDS-null or wild-type sperm. The above studies confirm the critical roles of GAPDHS and glycolysis in multiple steps of fertilization and suggest that sperm-specific glycolytic enzymes may be excellent targets for contraceptive development.

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EPIDIDYMOSOMES AND UTEROSOMES: THEIR ROLE IN SPERM UPTAKE OF GPI-LINKED PROTEINS

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Introduction: Epididymal and uterine luminal fluids (ELF and ULF, respectively) are the environments in which sperm undergo maturation and capacitation. In these fluids, sperm acquire multiple proteins necessary for fertilization. When luminal fluids are fractionated by ultracentrifugation, the pelleted fraction contains membranous vesicles known as uterosomes and epididymosomes. Like prostasomes, epididymosomes have been implicated in sperm uptake of glycosyl-phosphatidylinositol (GPI)-linked proteins. Recently, uterosomes have also been shown to transfer multiple GPI-linked proteins to the sperm surface via incubation, including sperm adhesion molecule 1 (SPAM1) a well characterized hyaluronidase known to play several roles in fertilization. This acquisition is negated when vesicles were subjected to PI-PLC treatment prior to incubation with sperm, indicating that an intact GPI anchor is necessary for SPAM1 uptake. When uterosomes and epididymosomes were visualized via TEM, immunogold labeling localized SPAM1 to the outer surface of these vesicles.
Objective: We sought to characterize the mechanism/localization of GPI-linked protein acquisition from epididymosomes and uterosomes by FM4-64FX labeling, confocal and transmission electron microscopy (TEM). A time course analysis comparing SPAM1 uptake to vesicular uptake was also performed in an attempt to determine if SPAM1 transfer from these vesicles occurs by vesicular docking and/or fusion.
Methods: Ultracentrifugation was used to pellet vesicles, which were then subjected to FM4-64FX labeling. After several washes, sperm were incubated in the presence of labeled vesicles. Both TEM and confocal microscopy were used to visualize vesicular binding on the sperm surface. Sperm incubated in labeled vesicles were fixed at various time points, immunostained for SPAM1 and subjected to flow cytometry to determine if the rate of SPAM1 uptake correlates with vesicular uptake.
Results Obtained: Incubation of sperm with FM4-64FX labeled vesicles revealed transfer of the label to the sperm plasma membrane (PM), indicative of vesicular docking. The acquired label was specifically localized to the PM overlying the acrosome and midpiece of the tail. Time course analysis revealed that although the amount of label corresponding to SPAM1 acquired increased over time, the amount of label corresponding to the vesicles remained relatively constant.
Conclusions: Vesicular docking is occurring within particular regions of the sperm PM, namely the PM overlying the acrosome and midpiece of the tail, the same regions associated with lipid rafts and acquisition of GPI-linked proteins. SPAM1, and potentially with other hyases, is transferred to the sperm surface along with the vesicles, but its rate of acquisition shows a different time course profile than the labeled vesicles.

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STABILITY OF MEMBRANE ASSOCIATED á-L-FUCOSIDASE IN HUMAN SPERM CELLS

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Two novel isoforms of á-L-fucosidase are present in human semen. Our lab has recently shown á-L-fucosidase is cryptically held within the acrosomal compartment and enriched within the sperm equatorial segment. Increase in enzyme activity is apparent following permeabilization of the sperm plasma membrane. The occurrence of these novel isoforms is provocative. Sperm proteins potentially involved in sperm-egg interactions must maintain their functional integrity as they travel through the female reproductive tract. The goal of this project was to investigate the stability of membrane associated á-L-fucosidase in human sperm. Seminal plasma and Percoll® washed sperm cell populations were incubated for 72 hours at 37°C, 5% CO_2, 100% humidity. At various times during prolonged incubation, sperm cells were permeabilized with 0.01% Triton®X-100 and enzyme assays using the fluorogenic substrate 4-MU-fuc were performed to measure enzyme activity. Stability of seminal plasma and membrane associated á-L-fucosidase was determined. Seminal plasma á-L-fucosidase activity rapidly decreased within 24 hours incubation at 37°C, 5% CO_2, 100% humidity. Conversely, á-L-fucosidase activity from Percoll® washed cell populations persisted up to 72 hours incubation under the same conditions. Seminal plasma á-L-fucosidase is considerably less stable than the membrane associated isoform. Control assays containing 4-MU-fuc and HSM or 0.01% Triton®X-100 did not interfere with measurement of á-L-fucosidase activity. Data from these experiments support the notions that 1) membrane associated á-L-fucosidase is stable for extended periods of time, consistent with a possible role in sperm-egg interaction and 2) compartmentalization within the human sperm is key to preserving protein integrity.

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LOCALISATION OF p80, THE BOVINE PH-20, ON THE SPERM PLASMA AND ACROSOME MEMBRANE

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Introduction: PH-20 is a glycoprotein linked on the sperm plasma membrane and the inner acrosomal membrane by a GPI anchor. This protein is well known among mammals for its hyaluronidase activity (N-terminus) and its implication in the secondary binding between spermatozoa and the zona pellucida following the acrosome reaction (C-terminus). We identified and characterized the bovine PH-20 (p80) in our lab. According to the deduced amino acid sequence, a transmembrane domain separates these 2 functional domains. Moreover, differences in the protein localisation were observed by indirect immunofluorescence according to the permeabilisation method used to treat sperm.
Objectives: Since both domains are required for the function of PH-20, the objective of this project is to determine p80 location and orientation on the bovine sperm membrane in order to better understand the mechanisms of action of the protein.
Method: First, we characterize two antibodies, a commercial monoclonal antibody #203-7D10 that recognises p80 on its N-terminus and ap80, a polyclonal antibody that we produced against a polypeptide from the C-terminus of the protein. These two antibodies were next used to localise p80 protein by indirect immunofluorescence on live and on fixed bovine sperm that were permeabilised or not. We also use ap80 antibody to localise the protein on sperm by transmission electron microscopy.
Results: Using the antibody directed against the N-terminal portion of the protein, p80 is localized on the post-acrosomal portion of the head of live spermatozoa whereas no detection was made with ap80 antibody. In sperm fixed with formaldehyde and permeabilized with triton, p80 was still detected on the post-acrosomal portion of the head using the MAb against the N-terminus, although ap80 detected the protein in the anterior portion of the head. In spermatozoa fixed/permeabilized with methanol, both antibodies recognized p80 on the anterior portion of the head and the signal is strongly reduced on the post-acrosomal region of the head. Results from transmission electron microscopy with immunogold using the polyclonal ap80 localised the protein inside the membrane of the anterior head, between the plasma and acrosomal membranes.
Conclusion: There are two populations of p80, one localised on the post-acrosomal membrane with its hyaluronidase domain facing the extracellular medium, and the other located on the acrosome with its C-terminal extremity facing the acrosomal lumen.

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MALE SEXUAL FUNCTION/PROSTATE/TESTIS CANCER/CLINICAL UROLOGY

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TREATMENT TO A HARDNESS GOAL OF FULL RIGIDITY IN MEN WITH ERECTILE DYSFUNCTION IS ASSOCIATED WITH SIGNIFICANT IMPROVEMENTS IN EMOTIONAL WELL-BEING AND SATISFACTION

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Objective: Although improved erectile function has been a traditional goal for erectile dysfunction (ED) treatment, treatment to a hardness goal of full rigidity may be the optimal goal. To better define the potential benefits of treatment to a hardness goal of full rigidity, measures of emotional well-being and satisfaction were assessed in men with ED whose erections improved from hard enough for penetration but not completely hard (grade 3) to completely hard/ rigid (grade 4).
Methods: Flexible-dose Viagra (sildenafil citrate) treatment was assessed in a 6-week double-blind, placebo-controlled (DBPC) trial with 6-week open-label (OL) extension (N=307; 25, 50, or 100 mg) and in a 10-week OL trial (N=107; 50 or 100 mg). Respective mean (range) age was 45 (18–55) y and 53 [20•82] y, and ED duration was 2 (<1–21) y and 4 (<1•18) y. Assessments included the International Index of Erectile Function, the Self-Esteem And Relationship questionnaire, the Quality of Erection Questionnaire, and the Erectile Dysfunction Inventory of Treatment Satisfaction.
Results: 35 Viagra-treated and 3 placebo-treated men improved from a most frequently achieved (modal) erection hardness of grade 3 at baseline to modal grade 4 hardness at DBPC end of treatment (EOT); mean±SD EOT scores were significantly improved from baseline for all outcomes for Viagra-treated patients (P<0.0001). At OL EOT, 42 previous-Viagra and 44 previous-placebo patients who had baseline modal grade 3 erection hardness had achieved modal grade 4 erection hardness; mean ±SD EOT scores were significantly improved (P<0.05), with EOT mean scores e 85% of maximal values. Likewise, patients improving from baseline modal erection hardness grade 3 to modal grade 4 hardness at EOT in the 10-week OL study (n=18) also achieved significant improvements from baseline in all outcomes (P<0.01), with mean EOT scores approaching maximal values.
Conclusions: In men with ED, erectile function and measures of self-esteem, confidence, and satisfaction (ie, sexual and with erection quality, erection hardness, overall relationship with the sexual partner, and ED treatment) improve to near maximal values as erection hardness increases from grade 3 to grade 4 hardness, suggesting that treatment to a hardness goal of full rigidity should be the optimal goal of ED treatment.

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6 MONTH INTERIM ANALYSIS OF THE LONGITUDINAL EFFECTS ON PENILE OXYGEN SATURATION FROM A RANDOMIZED STUDY OF THE NIGHTLY USE OF INTRAURETHRAL ALPROSTADIL VS SILDENAFIL FOLLOWING NERVE SPARING RADICAL PROSTATECTOMY (NSRRP)

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Introduction: Early penile rehabilitation is increasingly recognized as an important part of the recovery of erectile function after NSRRP. Postoperative intracorporal and intraurethral alprostadil as well as oral sildenafil have been reported to improve recovery of erectile function. Enhanced penile oxygenation is believed to be an important factor in the observed beneficial effect. The purpose of this study was to examine the longitudinal effect of nightly sildenafil or intraurethral prostaglandin on flaccid penile oxygen saturation.
Methods: A subgroup of 33 men enrolled in a larger (70 men) randomized comparative 11 month penile rehabilitation trial of nightly alprostadil (MUSE®) 250 mcg vs sildenafil 50 mg, underwent penile oximetry preoperatively, and at 1.25, 3, and 6 months postoperatively. Corporal oximetry was performed with the Odissey tissue oximeter at five sites; right thigh, right corpora, glans penis, left corpora, and left thigh.
Results: All men were preoperatively potent and underwent bilateral NSRRP by two surgeons (HL and ST). Mean age was 55. Right and left thigh oximetry did not change significantly over the 6 month period. Corporal oximetry in the MUSE cohort increased over 6 months while there was no significant change in the sildenafil group ($p<0.005$). Penile oximetry measurements of right corpora and left corpora were significantly greater in MUSE vs sildenafil patients at 6 months.
Conclusion: Despite the short half life of intraurethral alprostadil, beneficial effects of nightly alprostadil on flaccid penile oximetry are seen at 3 and 6 months. Flaccid penile oximetry in the sildenafil cohort decreased in the same period. Longer follow up is ongoing.
MUSE® and Sildenafil provided by VIVUS, Mountain View, CA
Oximeter provided by VIOPTX, Fremont, CA

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PENILE PROSTHETIC INFECTIONS IN PATIENTS UNDERGOING MULTIPLE PROSTHETIC-RELATED OPERATIONS

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Introduction: Penile prosthesis placement continues to be a popular treatment option for erectile dysfunction, but infection remains a dreaded complication. Our objective is to identify the risk factors for infection in patients who undergo multiple penile prosthetic-related operations (PRO).
Methods: We performed a single surgeon, single institution, retrospective review of patients who had a PRO between January 2001 and June 2006. Of these, only patients with a history of more than one PRO were included. We recorded operative detail from each PRO, and patient-specific factors examined included age, race, and medical history related to their ED, such as diabetes mellitus (DM), cardiovascular disease, history of priapism, sickle-cell disease, spinal cord injury (SCI), and Peyronnie's disease. The association of operative and patient variables with infection risk was examined using multiple logistic regression for dichotomous variables, and multiple linear regression for interval and ratio variables. The Human Assurance Committee approved the study.
Results: We identified 50 men, including 32 (64%) Caucasians and 18 (36%) African-Americans, with a median age of 60 (range 30 - 78) years. Comorbidities were DM in 30%, cardiovascular disease in 38%, and SCI in 8%. There were 9 patients (18%) with Peyronnie's disease, and 5 patients (10%) with a history of prolonged or recurrent priapism, including three patients with sickle cell disease. Each patient underwent a median of 3 (range 2 – 14) PROs, and the majority (74%) had penile prosthetic surgery before their referral to our institution. Significant noninfectious complications included eroded cylinder in two cases, exposed tubing requiring revision twice in one patient, and urinary tract injury in three cases. Five (10%) patients experienced a total of 7 prosthetic infections following an operation at our center, for an operative incidence of 7.6%. Significant predictors of prosthetic infections at our center included DM, SCI, younger age, and history of priapism. On multivariate analysis, however, only priapism (odds ratio 32, $p = 0.003$) remained significant. There was no significant effect of the number of previous PROs ($p = 0.47$), postoperative antibiotic prophylaxis with regard to duration ($p = 0.61$) or antibiotic choice ($p = 0.414$). Both history of priapism and Peyronnie's disease were associated with fewer PROs ($p = 0.008$ and $p = 0.005$, respectively). Of the patients with history of priapism, 3 (60%) currently have functioning prostheses at a mean follow-up of 21 months.

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CLINICAL SAFETY, HISTOLOGIC FINDINGS AND SURGICAL METHODS IN COMPLEX PHALLOPLASTY USING TYPE I COLLAGEN

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Objective : There may be a difference in effects and onset of complications according to the grafts as well as in the surgical technique for penile augmentation surgery. Recently, Type I collagen implant has been used in surgery and the safety has been improved and surgical techniques have been developed.
Methods: Type I collagen derived from bovine pericardium (Lyoplant®) was used for surgery. It has micro porous fibrous structure for rapid and regular ingrowth of autologous connective tissue. We have performed complex phalloplasty with minimal incision that enables simultaneous surgeries for glans

augmentation, penile lengthening, and girth enhancement.. Implant was designed according to the size of the penis. By dissecting the matrix with a knife horizontally and vertically to form a multi-layered slit or dividing the matrix into multiple pieces.
Results: The surgery was conducted on 550 patients who had small penis complexes from May 2003 to May 2006. The average augmented effect in circumference was 32 % and showed a mean length increase of 2.1 cm after the surgery. After implantation graft tissue is continuously replaced by endogenous connective tissue in histologic findings.
Conclusions: This complex phalloplasty using type I collagen implant with minimal incision showed positive results in terms of safety and effectiveness. Using the above-described technique will improve safety and recovery from the surgery, as well as the satisfaction obtained with the surgery.

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ADMINISTRATION OF PHOSPHODIESTERASE 5 INHIBITORS IN MEN WITH NON-INFLAMMATORY CHRONIC PELVIC PAIN
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We evaluated the effects of administration of phosphodiesterase 5 (PDE5) inhibitors in men with non-inflammatory chronic pelvic pain (NICPP).
All participants of the current study (group A; n=36) were men suffering from NICPP. All men had undergone cystoscopy and urodynamic evaluation. The Meares-Stamey test and urine cytology had been performed, as well. Each man was treated with an alpha blocker (aB) for at least 3 months. Every month, each man answered a questionnaire calculating a pelvic pain/discomfort score (PPDS; from 0 to 10; the grade 10 indicated a severe pain). After several months of treatment with the aB each man underwent a transrectal Doppler ultrasonography and a perineal prostatic biopsy (PPB). Then a number of men of group A (group B; n=20) were administered (in addition to the aB) sildenafil (50 mg per day; every day). The remaining men of group A received in addition to the aB vardenafil (20 mg per day; group C; n=16). Eight weeks after the combined treatment with aB and PDE5 inhibitors all men completed again the questionnaire and underwent a new transrectal Doppler ultrasonography and PPB. The materials of the first and second PPB were processed for Western immunoblot of phospho-eNOS (Ser-1177) and eNOS. In addition in the material of the first and second PPB we evaluated the total nitrite and nitrate content (an index of total nitric oxide production) and the malonaldehyde content (a marker of lipid peroxidation). Wilcoxon test was employed for statistical analysis. A probability P smaller than 0.05 was considered to be significant.
Within group B and within group C the mean values of PPDS were significantly smaller after the addition of the PDE5 inhibitor to the treatment protocol than prior to the sildenafil or vardenafil administration. In addition, within group B or C, phospho-eNOS and the mean value of the total amount of nitrite and nitrate in the PPB material were significantly elevated after the administration of PDE5 inhibitor. Prostatic arterial blood flow significantly increased after the administration of the respective PDE5 inhibitor within group B and within group C. In contrast, within the group B or C, the malonaldehyde content was significantly lower in the PPB material collected after the combined treatment with PDE5 inhibitor and aB compared with the first PPB.
The addition of PDE5 inhibitors to aB in the treatment of men with NICPP reduces quantitatively the PPDS probably by a) increasing eNOS activation in the prostate and resulting in an elevation of prostatic arterial blood flow, and b) decreasing lipid peroxidation in the prostatic cells. An upregulation in eNOS activation in PPB after the administration of PDE5 inhibitor is further supported by the increase in prostatic nitrite and nitrate content. This study suggests that PDE5 inhibitors may have a beneficial role in the management of men with NICPP.

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EFFECTS OF VARDENAFIL ON SPERM PARAMETERS AND SEMEN BIOCHEMISTRY
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Vardenafil is a commonly used pharmaceutical agent for the management of erectile dysfunction. We evaluated the effects of vardenafil on sperm physiology and semen biochemistry.
Eighteen infertile men participating in an assisted reproduction program were treated with vardenafil (10 mg every day) for at least 45 days. All these men had previously undergone at least one in vitro fertilization trial
without success. Prior to and after the 45-day-vardenafil-treatment six semen samples from each man were collected and evaluated for the standard parameters of semen analysis and for specific biochemical factors.
The total number of spermatozoa per ejaculate, the percentage of motile spermatozoa, the qualitative sperm motility, and the percentage of morphologically normal spermatozoa were significantly larger (P smaller than 0.05) in the semen samples collected after vardenafil treatment than in samples collected prior to vardenafil treatment (under the same period of sexual abstinence). In addition the citrate concentration in seminal plasma and the acid phosphatase concentration in seminal plasma were significantly larger in the samples collected after vardenafil treatment. There were no significant differences in fructose concentration, and alpha-glucosidase in the seminal plasma between samples collected prior to vardenafil treatment and those collected after vardenafil treatment. Four couples achieved pregnancies (in vitro fertilization techniques were employed) after vardenafil treatment.
Vardenafil administration in infertile men stimulating the prostatic secretory function increases the quantitative and qualitative motility of spermatozoa. In addition the larger total number of spermatozoa per ejaculate in samples collected after the vardenafil treatment may be due to enhanced contractions of the male genital ducts during ejaculation in men treated with vardenafil.

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CYTOKINES MCP-1, MIF AND ENA-78 IN SEMINAL PLASMA: RELATIONSHIPS WITH INFLAMMATION AND CHRONIC PELVIC PAIN SYNDROME

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Inflammation of the prostate or other male reproductive glands is associated with sperm dysfunction and is present in many men with chronic prostatitis / chronic pelvic pain syndrome (CPPS). Pain symptoms in CPPS may be mediated by leukocyte-induced oxidative damage, even though leukocytes are not evident in prostatic secretions or semen of all subjects. Cytokine and chemokine levels in reproductive fluids may offer a more sensitive and reliable measure of leukocyte activity. Among infertility and CPPS men, an expected relationship exists between pro-inflammatory cytokines in seminal plasma and leukocytospermia or prostatic secretion inflammation. However, a biomarker for CPPS is useful only if it can distinguish men with pain (whether or not with inflammation) from men without pain. Our goal is to identify such a biomarker.

Methods: Studies were approved by UW IRB. Previously frozen seminal plasmas from four groups of men were analyzed using validated ELISA methods for Monocyte Chemotactic Protein 1 (MCP-1), Macrophage Migration Inhibitory Factor (MIF), and Epithelial Neutrophil Activating peptide 78 (ENA-78). ELISA assays were validated for use in seminal plasma by linearity, spike recovery and Western blotting. The four groups were: Controls (**CTL**) without pain or inflammation; NIH category **IV**, asymptomatic controls with prostatic inflammation; NIH **IIIA**, CPPS subjects with inflammation; and NIH **IIIB**, CPPS subjects without inflammation. None had urogenital inflection, surgery or abnormalities, nor were any taking anti-microbial or anti-inflammatory medications at the time of evaluation.

Results: MCP-1 levels were significantly higher in IIIA and IIIB CPPS subjects (10.41 ng/ml, N=37) compared to CTL and IV controls (4.47 ng/ml, N=42) ($p=0.0005$, Mann-Whitney U test). MIF and ENA-78 concentrations were not significantly different between these combined groups. ENA-78 was elevated 59x ($p<0.005$) in IIIA and IV compared to IIIB and CTL, while MCP-1 and MIF exhibited no differences between inflamed *vs.* non-inflamed groups.

Conclusions: Although most cytokines studied to date show either no relationship with CPPS (*e.g.*, MIF), or are highly correlated with inflammation regardless of pain (*e.g.*, ENA-78), MCP-1 is elevated in CPPS subjects relative to pain-free controls, regardless of detected inflammation. Therefore, MCP-1 is a candidate biomarker for CPPS.

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EPIDIDYMIS

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CHARACTERIZING THE BIOLOGICAL FUNCTION OF CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PROTEIN USING A KNOCKOUT MOUSE MODEL

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The CRES protein is the defining member of a new subgroup within the family 2 cystatins of cysteine protease inhibitors. CRES is structurally related to the family 2 cystatins but lacks two of the three consensus sites necessary for cysteine protease inhibition and exhibits expression in the reproductive and neuroendocrine systems, specifically in caput epididymides, round and elongating spermatids, sperm acrosomes, corpora lutea, and anterior pituitary gonadotrophs. *In vitro*, CRES does not inhibit cysteine proteases, but rather, highly substrate-specific serine proteases called prohormone convertases which cleave inactive precursor proteins at mono- or dibasic sites to produce their mature active forms. Thus, CRES could be an important regulator of proteolytic processing necessary for reproduction. To test the biological role of CRES *in vivo*, CRES-null mice were generated. *In vivo* breeding, including examination of litter size, time to mating, and number of productive matings, suggested no difference in reproductive ability between CRES male and female wild-type (+/+) and null (-/-) mice. However, *in vitro* fertilization experiments using cumulus-oocyte complexes from CD-1 mice showed that sperm from CRES -/- mice were significantly less fertile than +/+ sperm (19% vs. 66% fertilized oocytes, $p<0.01$). Furthermore, sperm binding experiments demonstrated that CRES -/- sperm showed a 53% reduction in binding to zona pellucida-intact oocytes compared to +/+ sperm ($p<0.01$). The decrease in sperm-zona pellucida binding ability could reflect changes in earlier processes necessary for fertilization such as sperm motility and/or capacitation. Alternatively, the reduced binding may represent alterations in molecules on the surface of CRES -/- sperm. Proteomic analysis using two-dimensional difference gel electrophoresis (2D-DIGE) to compare CRES +/+ and -/- cauda sperm identified several proteins with altered levels including superoxide dismutase, mitochondrial glycerol-3-phosphate dehydrogenase, five different isoforms of phosphoglycerate kinase 2, alpha-tropomyosin, and myosin regulatory light chain 2. The 2D-DIGE results will be further confirmed by Western blot analysis. Taken together, these observations support a role for CRES in reproduction and sperm function.

Supported by NIH HD33903 (GAC).

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INVOLVEMENT OF ANGIOTENSIN II TYPE 2 RECEPTOR (AT2) IN VACUOLAR H^+-ATPASE (V-ATPASE) APICAL INSERTION AND V-ATPASE-DEPENDENT PROTON SECRETION IN EPIDIDYMAL CLEAR CELLS

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Angiotensin type 1 and 2 receptors (AT1 and AT2, respectively) are members of the G-protein coupled receptor family. Both AT1 and AT2 have been detected in the epididymis. While AT1 plays a role in electrolyte and fluid homeostasis, the role of AT2 remains elusive. We have shown that V-ATPase localizes in the apical pole of clear cells, and is critical for luminal acidification. In these cells, V-ATPase-dependent proton secretion correlates with V-ATPase apical membrane accumulation and extension of microvilli. In this study, we investigated the role of angiotensin II in these processes. Rat cauda epididymides were perfused *in vivo* with phosphate buffer (10 mM, pH 6.6) in the presence or absence of AngII (1 µM) for 20 min, followed by confocal microscopy analysis using an anti-V-ATPase antibody. Results showed that AngII significantly increased the surface occupied by V-ATPase-labeled microvilli by 58 ± 6% (n=4, $P<0.01$) compared to control (n=7). The AngII-induced V-ATPase apical accumulation was abolished by the AT2 antagonist PD123319 (1 µM), but not the AT1 antagonist losartan (1 µM). We further investigated the effect of cGMP, a downstream effector of AT2 receptor, on these processes. The cell permeable analogue of cGMP, pCPT-cGMP (1 mM), markedly increased the surface occupied by V-ATPase-labeled microvilli by 56 ± 34% (n=4, $P<0.05$). Similarly, the nitric oxide donor, sodium nitroprusside (1 mM), induced a significant elongation of V-ATPase-labeled microvilli by 44 ± 14% (n=3, $P<0.01$). Using a self-referencing proton-selective electrode on cut-open vas deferens, we showed an increase of concanamycin-dependent proton secretion by 45 ± 28% (n=3) after addition of AngII (1 µM). In summary, activation of AT2 receptor by luminal AngII induces the accumulation of V-ATPase into well-developed microvilli and increases V-ATPase-dependent proton secretion, probably via a cGMP-dependent pathway. Further studies will be required to determine whether activation of AT2 receptor generates the precursor of cGMP, nitric oxide, in clear cells.
This work is supported by NIH grants HD40793, DK38452 and P41R001395.

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ANDROGEN REGULATION OF APOPTOSIS AND SURVIVAL IN THE EPIDIDYMIS

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The epididymis is responsible for the proper maturation and storage of spermatozoa. It is separated into the initial segment (IS), caput (Ca), corpus (Co) and cauda (Cd) regions. Androgen withdrawal causes a segment-specific and time-dependent wave of apoptosis along the tissue, but the absolute number of apoptotic cells is small. Our aim was to investigate the early response of survival and apoptosis genes after the withdrawal and/or immediate replacement of androgen on the different segments of the epididymis. Five groups (n=5/group) of male BN rats were sham-operated or orchidectomized (ORC) and treated with either empty or testosterone (T)-filled implants designed to maintain control serum T concentrations. At 12 hrs and 1 day after treatment, rats were euthanized and epididymides separated into IS, Ca, Co and Cd for RNA extraction. One hundred biotinylated-cRNA samples were hybridized to SuperArray rat apoptosis-specific oligo arrays and analyzed using GeneSpring. Comparisons were done between sham-operated and ORC with empty or T-filled implants at each time point for each individual segment. The lists of differentially expressed genes were used to generate pathways with PathwayStudio. Immunohistochemistry was done to localize proteins to specific cell types. In the absence of T, 6% to 16% of the genes were differentially expressed; the most affected regions were the Co and Cd, at 12 hrs and 1 day, respectively. T maintenance partially prevented differential expression of genes, with only 3% to 9% of the genes remaining differentially expressed. Two genes, previously unidentified in the rat epididymis, Tnfrsf11b (osteoprotegerin, a tumor necrosis factor receptor) and Birc5 (survivin, an inhibitor of apoptosis), showed novel regulation; both were localized specifically to principal cells of the epididymis. Tnfrsf11b was most highly expressed in the IS, whereas Birc5 showed an increase in expression from proximal to distal regions. Tnfrsf11b was up-regulated after androgen withdrawal in all segments except the IS, where it was down-regulated, suggesting that its expression in the IS is dependent on testicular factors. After ORC, Birc5 was down-regulated in all segments and was maintained in the control range by T only in the Co and Cd, suggesting that testicular factors regulate Birc5 expression in the IS and Ca, whereas T regulates Birc5 expression in the distal epididymis. Pathway analysis pointed to basic fibroblast growth factor (FGF2), a testicular factor proposed as a regulator of the epididymis, as a potential regulator of the expression of both Tnfrsf11b and Birc5. The identification of specific apoptotic and survival genes affected by androgen withdrawal provides new insights into the molecular mechanisms underlying androgen regulation in the epididymis.

Supported by CIHR.

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NEW INSIGHTS IN SPERM ACQUISITION OF GPI-LINKED PROTEINS DURING EPIDIDYMAL MATURATION AND CAPACITATION: A ROLE FOR CLUSTERIN/APOJ

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Introduction: Previously we reported that Sperm adhesion molecule 1 (SPAM1), among other glycosyl phosphatidylinositol-(GPI)-linked proteins, can be acquired on the sperm surface *in vitro* from both the vesicular and the soluble membrane-free components of mouse epididymal luminal fluid (ELF) and uterine luminal fluid (ULF). Uptake is more efficient from the soluble component which, when sub-fractionated (ultracentrifugation, 230,000 x g), revealed the presence of insoluble oligomeric aggregates and predominantly soluble SPAM1 monomers (67 kDa). Since GPI-linked proteins are acquired during epididymal maturation and capacitation in the female tract, we postulate that there may be a common mechanism involved in their uptake from the soluble monomers. Based on our previous finding that SPAM1 uptake from this fraction is modulated by the presence of exogenous lipoproteins, we have proposed a novel role for Clusterin/ApoJ which is a well-known lipid transport protein that is abundantly present in the LFs. **Objective:** Our goal was to test the hypothesis that ApoJ, thought to play a role in cholesterol efflux from the sperm plasma membrane (PM), is responsible for stabilizing soluble GPI-linked monomers and facilitating their insertion into the sperm PM.
Methods: Sperm binding assays, antibody inhibition, and flow cytometric techniques were used to determine the effect of ApoJ on caudal mouse sperm uptake of SPAM1 from the membrane-free monomeric fraction of ELF and ULF. Co-immunoprecipitation was used to investigate SPAM1 and ApoJ interaction in the fraction.
Results: Antibody-inhibition of ApoJ in the soluble monomeric sub-fractions markedly reduced SPAM1 uptake. We also show for the first time an association of SPAM1 and ApoJ in co-immuno-precipitations, reflecting their interaction.
Conclusions: We present a model in which ApoJ in ELF and ULF stabilizes monomers of GPI-linked proteins and transports them to the sperm PM where they are inserted during epididymal maturation and capacitation. This model extends the currently held view that in cholesterol efflux at the sperm PM, lipid-poor ApoJ accepts cholesterol and transports it to the epididymal and uterine epithelial membranes for receptor-mediated endocytosis. Our work shows a novel role for ApoJ whose exact function has been an enigma for some time. It also has the potential of leading to advances in technology for the delivery of biomedically relevant membrane-free GPI-linked molecules to the sperm surface before artificial insemination or IVF, to enhance sperm maturation and function.

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CRES (CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC) PROTEIN IS AMYLOIDOGENIC

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The abnormal accumulation of aggregated protein, also known as amyloid, is associated with degenerative diseases including Alzheimer's and Parkinson's disease. An abnormal accumulation of a mutant form of cystatin C forms amyloid in cerebral arteries of patients with amyloid angiopathy. Previously, disease was thought to result from the mechanical disruption of cell function due to the presence of insoluble protein aggregates. However, accumulating evidence indicates that soluble oligomeric forms of proteins, precursors to amyloid, are cytotoxic and may be the causative form. Because of the active secretion of proteins and profound removal of fluid by the epithelium, macromolecular crowding is likely to occur in the tubular lumen of the epididymis causing amyloid-type protein aggregation. However, because of the critical role of the epididymis in sperm maturation, it is likely that surveillance/clearance mechanisms are in place to control this process and prevent the accumulation of cytotoxic protein structures. To date little is known regarding protein aggregation in the epididymis including mechanisms of formation, biological significance, or mechanisms of control. Our previous studies indicated that CRES formed high molecular mass soluble oligomeric structures in the epididymal fluid. To investigate whether CRES oligomerizes by the amyloidogenic pathway, recombinant CRES protein was incubated for prolonged times at 37°C and its structure examined by negative stain electron microscopy. CRES formed soluble oligomeric structures that advanced to protofibrils and eventually fibrils that stained with Congo Red characteristic of amyloid. Dot blot analysis using a conformation-dependent antibody further demonstrated that CRES formed soluble oligomeric structures typical of the amyloidogenic pathway. Preliminary studies of mouse epididymal fluid including the use of conformation-dependent antibodies, Congo Red, and in vitro cell culture cytotoxicity assays suggest that amyloidogenesis occurs in the epididymal lumen. Studies are ongoing to determine the role of CRES in this process and the biological significance of these oligomeric structures.

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MONDAY, APRIL 23, 2007
10:45 a.m. – 12:30 p.m.

Poster Session II
Location: Regency Ballroom 1-3

ANDROGENS/ENDOCRINOLOGY

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TESTOSTERONE PRODUCTION OF ISOLATED LEYDIG CELLS IN XXY MICE

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Introduction: Klinefelter's syndrome (KS) is the most common sex chromosome aneuploidy and affects approximately 0.5 % of the population. Our center has developed a mouse model that has phenotypic similarities with human XXY counterparts, presenting a unique opportunity to study the molecular mechanisms of Klinefelter's syndrome. The XXY mice also have hypergonadotropic hypogonadism typically found in the disorder which is highly suggestive of a Leydig cell steroidogenic defect. We have demonstrated that the Leydig cells in XXY mouse testes are both hypertrophic and hyperplastic, similar to men with Klinefelter's syndrome. Our study objective was to determine Leydig cell production of T in vitro in XXY mice as compared to XY mice.
Methods: Leydig cells from 7 adult XXY and 7 XY littermate mice were isolated. Aliquots of 20,000 pooled XY or XXY Leydig cells were cultured in each well, respectively. A total of 9 wells of XXY and 40 wells of XY Leydig cells were used in this study. Groups of 5 wells XXY or 20 wells XY Leydig cells were cultured for 3 hours at 37C. The remaining wells from each group received high dose (100ng/ml) LH stimulation. The supernatant testosterone concentration was measured by a specific radioimmunoassay.
Results: We showed a 2.5-fold decrease in XXY production of T (XXY 107.9± 29.2 ng/dl/105 cells) as compared to XY (265.2 ± 94.3ng/dl/105 cells) in non stimulated Leydig cells. In response to in vitro high dose LH 100ng/ml) stimulation, the T production in XXY (1287.1± 129.8 ng/dl/105 cells) appeared decreased in comparison to XY Leydig cells (1524.9 ± 228.3 ng/dl/105 cells). Thus, we demonstrated the testosterone production in XXY Leydig cells was impaired as in their human counterparts with Klinefelter's syndrome. To gain greater insight into the disorder of T production in XXY aneuploidy, we currently are performing more detailed dose response curves. In the future we plan to study steroidogenic enzymes expression and activity, and unravel responsible X-linked and regulated molecules which are important in understanding hypogonadism in Klinefelter's syndrome.

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IDENTIFICATION OF GALANIN RECEPTOR 2 IN RAT LEYDIG CELLS

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Galanin is a biologically active neuropeptide, widely distributed in the central and peripheral nervous systems and the endocrine system. Galanin acts via three distinct receptor subtypes Galr1, Galr2 and Galr3. However, it has not been established which of the three receptor subtypes (if any) is present in Leydig cells or whether galanin plays a role in regulating testosterone production. Given that the Leydig cell lineage may have a neuroectodermal origin and be subject to regulation by neuropeptides, galanin was investigated in the present study. Purified rat Leydig cells from three distinct developmental stages were collected: progenitor at postnatal day (PND) 21, immature from PND 35, and adult from PND 90. In addition testes were harvested on PND 21, 35 and 90 from normal rats, or from rats that were treated with ethane dimethanesulfonate (EDS), an agent that selectively kills Leydig cells and induces their regeneration, on days 4, 7, 21, 35, 49, 56 and 90 after EDS administration. Total RNA was obtained and analyzed for the level of Leydig cell specific gene expression. In addition, immature Leydig cells purified on PND 35 were treated with galanin in vitro, with and without luteinizing hormone, for 24 hr, and testosterone (T) was measured in the spent medium by RIA. Of the three galanin receptors, only Galr2 was detected in the Leydig cell. Galr2 expression disappeared when the Leydig cells were destroyed by EDS administration and was undetectable from days 4 to 21 days after treatment returning with the appearance of regenerating, progenitor Leydig cells on day 21 post EDS. Galanin significantly inhibited basal T production with an IC50 of 66.6 nM. In summary, Galr2 is present in Leydig cells and galanin inhibits basal T in the nanomolar range, indicating regulation under physiological circumstances.

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ENCLOMIPHENE (ANDROXAL™) RAISES TESTOSTERONE IN MEN WITH LOW OR NORMAL TESTOSTERONE AND PROVIDES A MORE NORMAL DAILY RHYTHM

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Objectives: We had previously shown that enclomiophene citrate (Androxal™) raised total serum testosterone (TT) in men with secondary hypogonadism. Our intention was to determine the pharmacodynamic effects when administered for two weeks to men with low or normal testosterone.

Methods: The study was conducted at a phase I unit in Houston (Breco Research Ltd). At the screening visit, subjects underwent physical examination and blood was drawn for laboratory analysis and vital signs were recorded. After providing informed consent subjects received study medication and were instructed to take two 12.5 mg capsules PO daily for 2 weeks. Subjects returned to the clinic 7 days later for the determination of TT. Fourteen days after first receiving study medication, subjects returned to the clinic in the morning. The last dose of study drug was taken in the clinic and that was designated time 0. Subjects remained in the clinic for 24 hours and blood was drawn thirteen times over the next 24 hours. Subjects underwent physical examination, medication and adverse event review. Subjects returned to the clinic 28 days later for a follow-up visit. At that time, blood was drawn, vital signs, adverse events, and concomitant medications were assessed and recorded.
Results: Eleven men, 5 with low initial TT (<350 ng/d, mean = 328 ± 111 ng/dl) and 8 with normal TT (>350 ng/d, mean = 458 ± 127 ng/dl) were enrolled in the study. An increase in TT was observed in males following 14 days of treatment. The mean net increase in TT was 231 ng/dl and 339 ng/dl for the low and normal groups respectively with few excursions above the upper limits. After 14 days of enclomiphene, mean testosterone levels over a 24†hour sampling period were relatively similar with respect to C_{avg}. Subjects demonstrated a more normal daily rhythm of TT with peaks in the mornings and a trough 12 hours after administration. PSA levels did not change for the 11 men who were investigated with baseline values of 0.70 ± 0.37 ng/ml versus values at day 42 of 0.73 ± 0.28 (p = 0.52, paired t-test) and all men were within normal limits. The TT levels returned to baseline after 28 days.
Conclusions: Enclomiphene is safe and effective in men with low and normal testosterone. Of particular interest is the partial restoration of normal rhythm. Additional studies would be warranted in men with normal TT.

Support: This worked was supported by Repros Therapeutics, Inc.

SPERMATOGENESIS/STEROIDOGENESIS/TESTIS BIOLOGY

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11ß-HYDROXYSTEROID DEHYDROGENASE (11ß-HSD) ISOZYMES IN THE HUMAN TESTIS

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Introduction and Objectives: Regulatory mechanisms within Leydig cells ensure maintenance of normal testosterone (T) levels. Stress and other conditions that elevate cortisol, a glucocorticoid, lead to decreased
T biosynthesis. In rats, Leydig cells express two isoforms of a glucocorticoid-metabolizing enzyme, 11ß-HSD. We hypothesize that human Leydig cells also produce two isoforms of 11ß-HSD, important for control of intracellular glucocorticoid levels through oxidative inactivation, thereby modulating T levels. This study aims to confirm the presence of both isoforms of 11ß-HSD in the human testis (11ß-HSD1 and 11ß-HSD2) and to determine if fertility status in men is correlated with oxidative and/or reductive activities.
Methods: Human testis samples were assayed for steady-state mRNA levels of 11ß-HSD1 and 11ß-HSD2 using SYBR green product detection in real-time PCR. Normal controls were men with obstructive azoospermia but normal sperm production. Comparisons were made among normal controls and those with nonobstructive azoospermia secondary to hypospermatogenesis, Sertoli cell only, or maturation arrest. Commercially purchased human placenta total RNA and total RNA extracted from human testis biopsies were used as a template to synthesize cDNA. Human placenta cDNA was used to construct relative standard curves for each amplicon in each experiment. Patient samples were measured in three replicates per experiment. Quantities of 11ß-HSD1 and 11ß-HSD2 mRNAs and 18S rRNA were calculated by extrapolation of cycle-threshold values on the standard curves. The calibrator sample contained a mixture of cDNA from four obstructed azoospermia (normal) samples.
Results: For all samples taken together, the median value of 11ß-HSD1 is 0.035 and of 11ß-HSD2 is 0.001, a statistically-significant difference ($p<0.0001$). There were no statistically significant differences noted in comparisons among the various pathologic groups.
Conclusions: 11ß-HSD1 and 11ß-HSD2 mRNAs are detected in human testis biopsy samples. 11ß-HSD1 levels are significantly higher than 11ß-HSD2 levels in the human testis. Further studies are needed to determine if 11ß-HSD1 and 11ß-HSD2 levels are related to spermatogenic status of the testis and to ascertain if the human testis is a site for glucocorticoid activity.

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ELMO INCREASES ENGULFMENT IN SERTOLI CELLS

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Studies in C. elegans identified *ced-5*, *ced-12*, and *ced-10* as components of a genetic pathway responsible for the engulfment of apoptotic cells. Subsequent work in mammalian cells have showed that the mammalian homologs of these genes, Dock180, ELMO, and Rac also play a critical role in engulfment of apoptotic cells. Dock180 and ELMO proteins function as a novel type of activator of the small GTPase Rac, that, in turn, constitutes an intracellular pathway that leads to actin polymerization and the formation of lamellipodia necessary for engulfment. Studies have also suggested that failure to promptly engulf dying apoptotic cells can lead to abnormal/altered development, as well as to long-term consequences such as autoimmunity. In the present study we investigated if the evolutionarily conserved protein ELMO (EnguLfment and cell MOtility) influences the ability of Sertoli cells to engulf surrogate apoptotic targets and apoptotic germ cells. Western blot and immunohistochemical analysis revealed that ELMO localized to Sertoli cells in the testis as well as the TM4 murine Sertoli cell line. In vitro engulfment assays employing the TM4 Sertoli cells with surrogate apoptotic targets (fluorescent microbeads) revealed that cells transfected with wild type (wt) ELMO had increased bead uptake compared to controls. wtELMO transfected TM4 Sertoli cells also had marked lamellipodia and readily engulfed apoptotic germ cells. Results from pull-down assays with an active-Rac binding protein revealed

that transfection with wtELMO lead to an increase in Rac activation. These data suggest that the evolutionarily conserved protein ELMO is in a pathway to Rac activation, actin polymerization, and the formation of lamellipodia in Sertoli cells which is necessary for the engulfment of apoptotic germ cells. Understanding the molecular mechanisms of how Sertoli cells clear apoptotic cell corpses will provide a better understanding of the role of Sertoli cells in the development of the seminiferous epithelium and in spermatogenesis. Moreover, the results of these studies will give novel insights into the pathology of such diseases as autoimmune orchitis and testicular cancer.

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ENHANCED cAMP-RESPONSIVE ELEMENT MODULATOR (CREM) in RAT TESTES TREATED by GINSENG RADIX

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Previous reports have shown Ginseng Radix (GR), the root of *Panax ginseng* to improve survival rate and sperm quality; inhibit lipid peroxidation, reduce radiation damage, protect against testicular toxicity. However, few studies have used rat testes to examine the effects of GR on male reproductive functions. In this study the relationship between GR and its effects associated with CREM gene expression and spermatogenesis *in vivo* has been examined. After Rats were treated GR for 56 days consecutively. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with GR ($p < 0.05$). The relative expression of CREM mRNA in the GR treated group was 174% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the GR treated group compared to the vehicle treated group ($p < 0.05$). The relative expression of CREM in the GR treated group was 108% than that in the normal group. In this study, Ginseng Radix (GR) has the enhancing effect of CREM expression in rat testes especially at mRNA level. These findings suggest that GR may have a role of improving male infertility related with the sperm alterations and proliferating or differentiating of germ cells closely related with CREM gene expression.

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YUKMIJIHWWANG-TANG AN ENHANCEMENT of CREM : ON REDUCED SPERMATOGENESIS by CYCLOPHOSPHAMIDE

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Yukmijihwang-tang (YMJHT) is a traditional medicinal formula that has been used for several hundred years. Cyclophosphamide (CP) is one of the chemotherapeutics that alter male fertile function. Even though YMJHT has been variously studied in Asian countries, rarely have its male reproductive functions been studied. In this study, the effects of YMJHT on the male reproductive functions were examined in rats with CP-induced testicular toxicity. Rats were treated CP for the first 7 days and YMJHT was treated for 56 days consecutively. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels in testes from the rats treated with CP have significantly decreased than those in testes from the rats treated with vehicle ($p < 0.01$), YMJHT treatment significantly recovered the decreased mRNA levels induced by CP by up to the normal value ($p < 0.01$). The relative expression levels of CREM in CP and YMJHT groups were 68 and 104% compared to that in normal group, respectively. The relative expressions of CREM in the CP and YMJHT groups were 77 and 102% than that in the normal group, respectively. These results demonstrate that Yukmijihwang-tang (YMJHT) has the recovering effects on reduced reproductive functions by chemotherapeutics, through the enhancement of spermatogenesis related with CREM expressions. Cervi Parvum Cornu (CPC), which is the velvet-like part of deer antlers has been used for thousands of years to enrich vital energy and to prolong life. Although several reports have attested to its aphrodisiac qualities, the relationship between CPC and the effects on male infertility have not been determined. Nor have the effects of CPC in regard to oxidative damage and spermatogenesis been established. Rats were treated CPC for 56 days consecutively. Animals were sacrificed and the testes were removed. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with CPC ($p < 0.05$). The relative expression of CREM mRNA in the CPC treated group was 115% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the CPC treated group compared to the vehicle treated group ($p < 0.05$). The relative expression of CREM in the CPC group was 144% than that in the normal group. Cervi Parvum Cornu (CPC) has an enhancing effect of CREM expression in rat testes both at mRNA and protein level. These findings suggest that CPC may have a role of improving male infertility related with the sperm alteration and proliferation or differentiation of germ cells closely related with CREM gene expression.

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RUBI FRUCTUS IS AN ENHANCEMENT OF cAMP-RESPONSIVE ELEMENT MODULATOR (CREM) IN RAT TESTES

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Rubi Fructus (RF), produce large amounts of antioxidants. Oxidative stress results from an imbalance between the production and removal of reactive oxygen species (ROS). ROS and nitric oxide (NO) has recently been associated with male infertility. Although research has examined many effects RF has on biological systems, the relationship between RF and its effect on male reproductive malfunction associated with oxidative damage has yet to be elucidated. Rats were treated RF for 56 days consecutively. Animals were sacrificed and the testes were removed. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with RF ($p < 0.05$). The relative expression of CREM mRNA in the RF treated group was 189% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the RF treated group compared to the vehicle treated group ($p < 0.05$). The relative expression of CREM in the RF group was 112% than that in the normal group. This result confirmed mRNA data. In conclusion, Rubi Fructus (RF) has an enhancing effect of CREM expression in rat testes both at mRNA and protein level. These results suggest that RF may have an effect on proliferation and differentiation of germ cells closely related with CREM gene expression.

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CORNI FRUCTUS INVOLVEMENT in EXPRESSION of cAMP-RESPONSIVE ELEMENT MODULATOR (CREM)

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Corni Fructus (CF), the fruit of Comus officinalis is also known by common names of Asiatic cornelian cherry and dogwood fruit. Recently, many studies have examined how CF affects anti-oxidative damage and spermatogenesis in vitro, the relationship between CF and its effects on male reproductive malfunction associated with CREM gene expression and spermatogenesis in vivo has not yet been elucidated. Rats were treated CF for 56 days consecutively. Animals were sacrificed and the testes were removed. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with CF ($p < 0.05$). The relative expression of CREM mRNA in the CF treated group was 153% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the CF treated group compared to the vehicle treated group ($p < 0.05$). The relative expression of CREM in the CF group was 132% than that in the normal group. Corni Fructus (CF) has an enhancing effect of CREM expression in rat testes both at mRNA and protein level. These findings suggest that CF may have a role of improving male infertility related with the sperm alteration and proliferation or differentiation of germ cells closely related with CREM gene expression.

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REPRODUCTIVE CHARACTERIZATION of CERVI PARVUM CORNU in cAMP-RESPONSIVE ELEMENT MODULATOR (CREM)

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Cervi Parvum Cornu (CPC), which is the velvet-like part of deer antlers has been used for thousands of years to enrich vital energy and to prolong life. Although several reports have attested to its aphrodisiac qualities, the relationship between CPC and the effects on male infertility have not been determined. Nor have the effects of CPC in regard to oxidative damage and spermatogenesis been established. Rats were treated CPC for 56 days consecutively. Animals were sacrificed and the testes were removed. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with CPC ($p < 0.05$). The relative expression of CREM mRNA in the CPC treated group was 115% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the CPC treated group compared to the vehicle treated group ($p < 0.05$). The relative expression of CREM in the CPC group was 144% than that in the normal group. Cervi Parvum Comu (CPC) has an enhancing effect of CREM expression in rat testes both at mRNA and protein level. These findings suggest that CPC may have a role of improving male infertility related with the sperm alteration and proliferation or differentiation of germ cells closely related with CREM gene expression.

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IMPROVED SPERM CHROMATIN DECONDENSATION RATES IN HAMSTER-OOCYTE ICSI AFTER ANNEXIN-V-MACS IN INFERTILITY PATIENTS

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The depletion of apoptotic sperm using Annexin-V based magnetic cell separation (MACS) has been recently introduced as a mean to improve assisted reproduction outcomes. Annexin-V MACS enhances hamster-oocyte sperm penetration but does not increase sperm chromatin decondensation (SCD) rates following hamster-oocyte ICSI (H-ICSI). Our aim was to evaluate the SCD rates of the annexin-negative (non-apoptotic) sperm fraction of patients with infertility using H-ICSI.
Semen specimens collected from 21 infertility patients with subnormal sperm parameters were subjected to double density gradient centrifugation (DGC) followed by Annexin-V MACS. A non-separated aliquot of each sample served as control. H-ICSI was performed in all aliquots using 20 frozen-thawed hamster oocytes per aliquot. Results were evaluated as the percentage of
oocytes showing SCD. In addition, caspase-3 activation (CP3) and disruption of transmembrane mitochondrial potential (TMP) were monitored by FACS to observe the separation effect.
Annexin-V MACS resulted in a significant enrichment of spermatozoa with inactive CP3 and intact TMP in the annexin-negative fraction. Similarly, annexin-negative spermatozoa had the highest SCD rates compared to controls and annexin-positive sperm (table).
In conclusion, semen samples from infertility patients contain high levels of spermatozoa with active CP3 and disrupted TMP. Compared to routine DGC the enrichment of non-apoptotic spermatozoa by Annexin-V MACS resulted in superior sperm chromatin decondensation and may be used to improve the outcome of ICSI procedures in infertility patients.

Parameter	Controls	Annexin-negative	Annexin-positive
active CP3 (% sperm)	43.5 ± 13.8	26.8 ± 12.3*	58.4 ±11.7*
intact TMP (% sperm)	54.7 ± 23.2	71.6 ± 21.5*	9.8 ±12.0*
SCD (% oocytes)	31.3 ± 13.1	44.2 ± 15.8*	18.3 ± 6.7*

Values are expressed as mean ± standard deviation. *p<0.01 in comparison to control. Statistical test: Wilcoxon-test.

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AF-2364[1-(2,4-DICHLOROBENZYL)-INDAZOLE-3-CARBOHYDRAZIDE] INHIBITS SPERMATOGENESIS IN MALE RABBITS

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AF-2364 [1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide], an analogue of lonidamine, has been shown to potently inhibit spermatogenesis when administered orally to adult Sprague-Dawley rats. The efficacy of the drug in other animals has not been evaluated. The effects on fertility efficacy, organ weights, and tissue morphology and plasma concentration of AF-2364 were examined in male Japanese rabbits. Six animals of each group were administered 25 mg/Kg AF2364 once a week for 2 weeks either by intravenous injection or gavage. Vehicle treated rabbits were used as controls. 7 and 14 days after treatment, testes were removed for optical and electronic microscopy. Bloods were drawn to measure plasma the concentrations of AF-2364 by HPLC. Testis showed rapid exfoliation of elongated spermatids and the generation of large multinucleated cells 7 days after the first treatment, with depletion of most germ cells after 14 days in both intravenous and oral treatment. Electron microscopy showed the Sertoli-germ cell adherens junctions were significantly disturbed in both treatments. The intravenous treatment showed much severe disturbance of Sertoli-germ junctions compared to gavage treatment, which was correlated with bioavailability of the drug. These results illustrate the potential of AF2364 as a male contraceptive and the efficacy is related with the bioavailability of the drug.

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REVERSIBLE ANTIFERTILITY EFFECT OF AQUEOUS LEAF EXTRACT OF *ALLAMANDA CATHARTICA* L. IN MALE MICE

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Currently, safe and reversible male contraceptives are not available. In the present study, we investigated the contraceptive potential, reversibility, and toxicity of aqueous leaf extract of *Allamanda cathartica* in the laboratory mouse [Parkes (P) strain]. Leaf extract was administered orally at 150 mg/kg BW/

day for 14, 28, and 42 days to male mice and the effects on body and organ weights, testicular histology, serum level of testosterone, sperm parameters, haematology, and serum microchemistry were evaluated. Fertility tests were also performed in mice administered 150 mg/kg body weight of the extract for 42 days, at intervals up to 56 days after cessation of the treatment. Histologically, testes of extract-treated mice showed non-uniform degenerative changes in the seminiferous tubules as both affected and normal tubules were observed in the same cross-sections; the frequency of degenerated tubules was significantly higher in treated mice compared to controls. Significant reductions were also noted in the height of the germinal epithelium and diameter of the seminiferous tubules in testes of treated mice compared to controls. Frequency of stages VII-VIII of the spermatogenic cycle was also markedly decreased in treated mice. Serum level of testosterone remained unaltered in treated mice. The treatment also had adverse effects on motility, viability, morphology, and number of spermatozoa in the cauda epididymidis and on the level of sialic acid in the epididymis. The treatment, however, had no effect on serum levels of ALT, AST, and creatinine, or on haematology and histoarchitecture of liver and kidney. Libido remained unaffected in treated mice, but there was a marked reduction in the fertility of treated males. Remarkably, 56 days following cessation of treatment, all the parameters studied in the present investigations recovered to control levels. The present results, thus, suggest that *A. cathartica* treatment causes reversible antifertility effect in male mice, without any apparent toxic manifestation.

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THE RELATIONSHIP BETWEEN SERUM LEVELS OF GONADOTROPINS, PROLACTIN, TESTOSTERONE AND ESTRADIOL IN SERTOLI CELL ONLY SYNDROME.

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To investigate whether an impaired Leydig cell function is present in Sertoli cell only syndrome (SCOS), serum luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), testosterone (T) and estradiol (E2) in 38 infertile SCOS men were compared to 7 men with normal spermatogenesis. In addition, the T/LH ratio and E2/T ratio were compared between two groups. Compared to control (mean ± SE, 19.0 ± 1.9 ng/DL), SCOS patients had significant lower serum testosterone level (13.6 ± 2.0 ng/DL). SCOS patients also had higher FSH level (23.3 ± 2.1 IU vs. control 7.0 ± 1.3 IU) and E2/T (11.1± 2.3 vs. control 8.3 ± 2.5), and lower T/LH ratio (2.1 ± 0.2 vs. control 3.73 ± 1.0). PRL levels of SCOS patients did not significantly change when compared to control. The present study showed the impaired Leydig cell function in SCOS patients.

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AGE AS A PREDICTOR FOR OOCYTE REPAIR CAPACITY FOR SPERM DNA DAMAGE

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Introduction and Objectives: The role of sperm DNA damage as an effector of ICSI outcome is now more clearly understood. However this effect is not sharply demarcated and variability of results is not explained. Female age is a well known contributing factor on ICSI results. That may be partially related to oocyte nuclear aging; impairing its DNA repairing capacity and acting as a regulator for sperm DNA damage effect on ICSI outcome. The objective of this study was to test the association between female age and the extent of DNA damage on ICSI outcome.
Methods: Twenty-one couples undergoing infertility treatment by ICSI were involved in the study. Sperm DNA damage was assessed by Comet assay and DNA damage was classified as mild, moderate and severe. The effect of female age on fertilization rate, % of grade A embryos in patients with partners having mild and moderate or severe DNA damage was analyzed
Results: Of the 21 couples, sperm from 3 male partners showed severe DNA damage and these were excluded from the analysis due to their small sample size. Female patients were divided according to age into 2 groups: Group I with patients < 32y and group II with patients' age > 32y. Twelve female patients were in group I, with mean age 27.3 ± 2.6, while 9 were in group II, with mean age of 34.4 ± 2.5. The mean fertilization rate was 80.1% in group I versus 69.5% for patients in group II and the embryo grade A% was 74.5 % vs. 52.4%. Patients in group I with male partners having mild DNA damage had fertilization rate of 82.5% and embryo grade A of 72.3%. Patients in group II with male partners having mild DNA damage had fertilization rate of 75.5% and embryo grade A of 51.3%. In couples with male partners with moderate DNA damage, group I patients had fertility rate and embryo grade A of 72.8% and 71%, while patients in group II showed 72.2% and 43.9% respectively.
Conclusion: Sperm DNA effects on ICSI outcome are female age dependent. In young age group, decline in fertilization rate is increasing with increase in sperm DNA damage, while embryo grade is not affected. In older women, although fertilization rate is not affected, embryo grade decreases with increase in sperm DNA damage. A larger sample size will be helpful in defining the exact algorithm combining female age and sperm DNA damage for prediction of success rates in ICSI.

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FACTORS AFFECTING THE RATE AND EXTENT OF SPERM OUTPUT SUPPRESSION IN MALE CONTRACEPTIVE PURPOSES

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Context: Male hormonal contraceptive methods rely on adequate suppression of sperm output. Factors predicting the rate and extent of sperm suppression are largely unknown.
Objective: To determine the potential modulating covariables that modify suppression of spermatogenesis to thresholds (< 3 or 1 M/mL) that are sufficient for contraceptive purposes.
Design: An integrated multivariate logistic and stepwise and best-subset Cox proportional hazards analysis of all published studies of at least 3 months treatment duration.
Setting: Deidentified individual subject data directly collected from individual investigators from 30 studies published 1990-2006.
Participants: 1647 normal (by physical, blood and semen exam) men aged 19-51 years of predominately Caucasian (two-thirds) or Asian (one-third) descent. This represents about 90% of all the published data.
Intervention(s): Men were treated with testosterone (T) with or without progestin for 3 to 18 months.
Results: Sperm density fell to less than 1 M/mL after a median time of 77 (74-82 95% CI) days (Kaplan-Meier), however about 5.8 (5.2-6.4) % of men who suppressed to 3 M/mL never suppressed to 1 M/mL. Multivariate logistic regression showed that higher testosterone dose was the single most important variable predicting less complete suppression of sperm output. Multivariate Cox analysis was used to examine suppression rates and showed faster suppression in Caucasians, depot testosterone preparations and higher testosterone dosage, the additional use of progestin therapy and lower initial blood testosterone concentration. Younger age, lower initial sperm concentration and lower total body weight were also associated with faster suppression, but the adjusted effect sizes were small. For a typical 35 year old Caucasian man treated with intramuscular injections of T undecanoate and any progestin, the median time to suppression of < 1 M/mL was 60 (58-65) days. These times were 84 (77-91) and 76 (67-84) days, if progestins were not coadministered, or if the man was Asian.
Conclusion: We have identified important predictors, and quantified expected probabilities that sperm output will be suppressed to concentrations sufficient for reliable contraceptive purposes. Not all men will suppress sperm output to levels compatible with reliable contraception. Paradoxically, therapies utilizing higher testosterone doses increased the rate and blunted the chance of more complete suppression of sperm output. These data are critical for the development and utilization of male hormonal contraceptive regimens.

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CORRELATION OF SPERM DNA DAMAGE AND PREGNANCY OUTCOME IN PATIENTS UNDERGOING ICSI

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Introduction and Objectives: Sperm DNA integrity is essential for the transmission of genetic information. Any form of sperm chromatin abnormalities or DNA damage may result in male infertility. Comet test can measure single and double strand breaks of DNA in spermatozoa. The aim of our study was to examine the predictive value of sperm DNA fragmentation by comet assay with ICSI outcome.
Methods: 21 semen samples of infertile patients undergoing ICSI were tested. Sperm DNA damage was analyzed by Comet test (single cell gel electrophoresis). DNA fragmentation was classified as mild, moderate and severe according to the tail moment.
Results: The mean ± SD for mild, moderate and severe DNA damage was 42.05 ± 15.52, 36.19 ± 8.43 and 20.62 ± 17.43 respectively. Significant differences ($P<0.001$) were seen between mild and severe DNA damage. When ICSI outcome was examined, high positive correlation was seen between mild DNA damage and pregnancy rate ($r = 0.495$; $P<0.001$). High negative correlation was also seen between severe DNA damage and pregnancy rate ($r = -0.485$; $P <0.001$). No significant correlation was found between moderate sperm DNA fragmentation and pregnancy rate.
Conclusions: Assessment of sperm DNA fragmentation by comet assay has a higher predictive value for ICSI outcome in mild and severe DNA damage but not in those with moderate damage.

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DIFFERENTIAL SPERM mRNA EXPRESSION PROFILE FROM FERTILE VS INFERTILE MALES: A PRELIMINARY MICROARRAY APPROACH OF SPERM EXPRESSION PROFILES (SEP) IN FERTILITY

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Introduction: Sperm analysis based on sperm count and motility has been employed for the diagnosis of male fertility for several decades. It is an easy, inexpensive and useful tool to determine the fertile status of a male. A significant number of infertile males are diagnosed with idiopathic infertility and all sperm parameters are normal. Recent investigations have described the sperm mRNA relevance in fertilization and early embryo development. Microarray technologies can inform about a wide range of mRNAs expression within a single experiment, and are ideal in analyzing the expression profiles in cells or tissues. Our aim was to compare the sperm expression profiles obtained from infertile males and compare with the fertile sperm donors by employing microarray technology and determine the differentially expressed genes that may be potentially involved in male fertility.
Material and methods: Sperm samples were obtained from infertile males (n=5) and proven fertile donors (n=5) with normal sperm count and motility (WHO criteria). Sperm mRNA was extracted using Trizol; and resuspended in DEPC-treated water and frozen at -80 until the microarray experiments were performed. RNAs from the same groups were pooled before the analysis. Human whole Genome bioarray contains more than 55,000 gene targets. Comparisons between the two groups were performed in duplicate. Intensities were normalized and analyzed using CodeLink Expression Analysis v4.1 software.
Results: Only differentially expressed genes that were expressed at least ten times compared with the control group were included. Our preliminary results confirm that within the differentially expressed genes there are few genes that are overexpressed (n=3), while all others are underexpressed (n=133) in infertile males. Our results suggest that failure to impregnate a woman with normal sperm production may be due to the lack of factors involved in correct sperm function.(Figure 1). Negative results denote a higher expression in the control group. Among all the gene sequences found to be differentially expressed, it is notable to remark the presence of several ribosomal proteins and factors involved in spermatogenesis. Interestingly, those spermatogenesis factors are not related with sperm production in terms of number of ejaculated spermatozoa given that all the samples obtained for his analysis were within normal range of sperm count and comparable in both groups.
Conclusions: This is the first time that a significant difference in the mRNA sperm expression profiles in spermatozoa obtained from infertile males vs spermatozoa obtained in fertile men with comparable sperm count has been described. These differences imply several molecules in a broad spectrum of biochemical and physiological pathways, at different points. These results confirm the complexity of the events involved in sperm function.

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A STUDY TO DETERMINE USING SHARED OOCYTES IF THE SPERM PER SE CAN BE THE CAUSE OF SLOW EMBRYO CLEAVAGE

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Recent data evaluating the effect of embryo blastomere number on pregnancy rates following single embryo transfer (ET) showed a large difference between day 3 4-cell and 5-cell embryos (3.8% and 9.5% per ET, respectively) vs. 6-8 cell embryos (38%, 40%, and 42.5%). Excessive embryo fragmentation has been shown in some instances to be related to the sperm and not the egg. This was demonstrated by a case report where a couple repeatedly made in the majority of their failed ET cycles highly fragmented embryos. However, when the female partner shared half of her eggs with another couple, despite the fact that she again made highly fragmented embryos, the other couple made 100% top quality embryos. These embryos made with another man's sperm resulted in delivered triplets with 3 of 3 embryos implanting and the 3 frozen embryos donated to another couple also resulted in triplets. By evaluating oocytes shared by 2 couples this study would try to determine if the sperm per se may be responsible for slow cleavage resulting in low blastomere number on day 3. Two sources of anonymous oocytes were used for sharing between 2 couples: paid donors and infertile women trying to conceive themselves. Sharing couples would be sought where there appeared to be a marked discrepancy between percentage of embryos with only 4 or 5 blastomeres on day 3 vs. 6-8 cells. If such a couple would be found the percentage of slow cleavage embryos would be evaluated in a subsequent donor egg cycle with determination if the same or a different source of oocytes were used. There were 376 paired cycles evaluated. Only 1 of 752 women failed to have any embryos with 6 or more blastomeres in her first IVF cycle. The 43 year old woman first had her own IVF cycle and had a 5 and 4 cell embryo transferred. She conceived but had a miscarriage. She then became a recipient. She transferred 4 embryos but all were 4 cells but her infertile donor had 3 embryos transferred 4, 6 and 8 cells. Despite slow cleavage the woman conceived and delivered healthy twins. Thus male factor does not appear to be a cause of slow embryo cleavage at least to the point of inhibiting any embryo from achieving 6 blastomeres. A sperm factor does not appear to be a common cause of slow cleavage rate of all embryos formed but possibly could still result in fewer fast cleaving embryos.

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ASSOCIATION OF FERTILITY POTENTIAL WITH TOTAL GENOME DAMAGE IN SPERMATOZOA

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Introduction and Objectives: Evaluation of sperm DNA damage is crucial in clinical practice as it impacts on reproductive outcomes. Detection of Total genome damage (DNA fragments) is based on a method introduced by Aljanabi and Martinez 1997. Our objective was to examine sperm total genome damage in proven fertile & infertile men.

Methods: Routine semen analysis was done for proven fertile men (n = 7) and infertile men (n = 13). Briefly, sperm pellets (3 X 10^4 cells) were treated for salting out DNA extraction & purification. Gel electrophoresis was performed for 2 hours at 50 volt. Gel photos were analyzed by Gel Pro Analyzer software version 3.1 Media Cybernetics, USA.

Results: Significant differences were seen in the maximum optical density (max OD) between fertile and infertile group in terms of the intact and damaged DNA (d"500bp) (P = 0.004 and 0.025 respectively). Max OD of the damaged DNA (500-1000bp) was comparable between the two groups.

Conclusion: Total Genome damage test can differentiate between fertile and infertile men. Infertile men show higher incidence of short DNA fragments in their semen.

Groups	Intact DNA	Damaged DNA	
		(500-100bp)	(≤500)
Proven Fertile (n = 7)	148.03±14.93	6.4±6.2	4.8±4.8
Infertile patients (n = 13)	75.07±14.29	18.2±9.9	105.69±29.8
P value	0.004	0.4	0.025
Results expressed as mean ± SEM, P <0.05 was considered significant using Mann Whitney test.			

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IDIOPATHIC MALE INFERTILITY: CLINICAL AND MOLECULAR PROFILE

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Idiopathic infertility is an enigma as the cause is still not clear. With the advances in molecular tools some of the pathophysiology is being unraveled. Genetic factors are being seen as one of the etiology causing male infertility. The role of the genes and their protein expression with regards to spermatogenesis is not clearly understood. FSH or Inhibin B alone cannot act as markers of spermatogenesis neither predict sperm retrievals during intra cytoplasmic sperm injection (ICSI). There is a concern that genetic aberrations are transmitted to the male progeny during ICSI and also regarding bone health and early andropause in idiopathic male infertility.

Objective was to study the hormonal profile, an a functional marker of spermatogenesis with special emphasis on the frequency of Y chromosome microdeletions, the phenotype/genotype association and the gene and protein expression in Nonobstructive azoospermic (NOA) and idiopathic severe oligozoospermic males (SOAS).We enrolled 150 Nonobstructive azoospermic (n=72) and severe oligozoospermic males (n=73) after screening 560 infertile males who attended the Institute's male infertility clinic. PCR using 6 STS primers for Yq microdeletions and serum FSH, LH, Testosterone, Inhibin B was estimated. Histological classifications of testicular biopsies, karyotyping, in- situ hybridization and immunohistochemistry for gene and protein expression respectively was carried out as and when tissue was available. Genetic and abnormal karyotype was seen in 4.2% and 4.6% subjects respectively. Frequent deletions were in the AZFb and AZFc regions. FSH and LH were significantly higher, serum inhibin B was significantly low and a more severe testicular phenotype was seen in NOA group. Men with Y deletions, showed lower testosterone levels. Large numbers of subjects are required to rule out a probability of early andropause and osteoporosis in this group. The mRNA of the DAZ gene showed a weak expression in the testicular biopsy of an infertile subject who did not have a microdeletion indicating that low expression of gene could lead to decreased spermatogenesis. With reference to the above observations, there is a need for screening and counseling of couples. Expression of genes and their proteins, the testicular growth factors and steroid hormones are interrelated in the complex cascade of spermatogenesis and any aberration in this interaction can lead to infertility.

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INITIAL EXPERIENCE WITH ROBOTIC VARICOCELECTOMY

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Introduction: Robot-assisted operations are gaining popularity in urological procedures. Microsurgical varicocelectomy is at the present time considered as the most effective and safest treatment for varicocele. Microsurgical operation can be difficult and should be used only by surgeons who perform the operation frequently. We are reporting our initial experience using robot-assisted varicocelectomy for the treatment of varicocele.
Patients and Methods: Three patients aged 17 to 28 with varicocele (two patients with left and one patient with bilateral diseases) underwent robot-assisted varicocelectomy after the patients were consulted in detail regarding this experimental procedure. All four varicocelectomies were performed through sub-inguinal incisions. The spermatic cord was exposed and delivered out of the wound with a Penrose drain underneath. The Da Vinci robot was then brought in and placed above the surgical field. The testicular artery and vas deferens with vasal artery and small vasal veins were identified and isolated. All other veins within the cord were ligated with 5-0 Vicryl sutures and divided. At the completion of the varicocelectomy, only the testicular artery, lymphatics, and vas deferens with its vessels remained. The surgical time and the difficulties of each step of the procedure were recorded. The surgical and post-operative complications as well as recovery time were analyzed.
Results: The average operative times were 90 ± 25 minutes (mean ± SD). The follow up time were from two weeks to 5 months. No intra-operative or post-operative complications were identified. No recurrence of varicocele was seen in patients (three varicocelectomies) within 5 month follow-up. There were no obvious difficulties with each step of the robotic procedures in identifying and isolating vessels and vas deferens. Tying the 5-0 sutures required a short learning curve due to the lack of tactile sensation with the robot. All patients resumed daily activities the same day of the surgery and full activities within two weeks.
Conclusions: Robot-assisted varicocelectomy can be safely and effectively performed without a slow learning curve. The cost-effectiveness and efficacy regarding the improvement of semen quality and pregnancy for patients with infertility need to be studied in a larger population.

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EVALUATION OF CRYOPRESERVATION PROTOCOLS AND REMOTE COLLECTION IN CANCER PATIENTS

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Patients diagnosed with cancer are often referred to sperm banks for sperm cryopreservation. These patients may have poor sperm quality, and after freezing and thawing, it is estimated that the sperm motility further decreases. Therefore, cryopreservation protocols should be properly selected to compensate for these defects. The objective of our study was to compare sperm cryosurvival in association with different cryopreservation protocols. Specimens were collected from 89 patients with testicular or systemic malignancy prior to the initiation of therapy. CryoFly® system was used to collect 67 specimens from remote locations overnight. Cryopreservation protocols used were: standard (raw sperm + cryoprotectant, n=177) and pre-washed (washed sperm + cryoprotectant, n=15). Sperm cryosurvival rate (CSR) was calculated by dividing motility post-thaw by motility pre-freeze X 100.
Sperm concentration was comparable in all specimens. CryoFly® specimens had significantly lower motility compared to standard preparations ($p<0.001$) but not pre-washed. CSR was significantly lower in CryoFly® specimens compared to standard preparations ($p<0.05$) but was significantly higher compared to pre-washed preparations ($p<0.001$).
In conclusion, standard preparation is the most optimum method for sperm cryopreservation in cancer patients. Sperm washing prior to cryopreservation should not be recommended. Although sperm motility is affected as a result of using CryoFly®, these specimens display cryopreservation tolerance. Components such as albumin in the CryoFly® kit could be the reason for the relatively high CSR.

Cryopreservation protocol	Specimens (n)	Concentration (Million/mL)	Motility (%)	CSR (%)
CryoFly®	67	48.4±52.4	33.1±16.1	50.7±2.8
Standard	177	48.2±4.2	47.3±1.1	43.1±1.3
Pre-washed	15	60.5±15.6	41.3±3.1	29.7±2.0

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SEMINIFEROUS TUBULES WITH AN ANOMALOUS OUTLINE IN THE TESTICULAR DYSGENESIS SYNDROME

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Introduction: The entity of Testicular dysgenesis syndrome includes several heterogenic pathologies as undescended testis, hypospadias, testis cancer and infertility. Associations of different testicular histological lesions have been observed in the testicular dysgenesis syndrome including undifferenciated seminiferous tubules, partial Sertoli-cell-only tubules, testicular microliths, CIS cells and Leydig-cell micronodules.
Material and Methods: In a retrospective revision of 450 prepubertal and adult patients with infertility, crytorchidism, intersex and testicular cancer from the files of La Paz University Hospital, 15 cases with seminiferous tubules with abnormal configuration were observed
Results: In all 15 cases a spectrum of different patterns of malformative configuration of the seminiferous tubules were seen. The morphological anomalies of the seminiferous tubules included frequent ramifications, annular configuration, culde sac dichotomy ending and changes in diameter in different segments, with dilatations alternating with hypoplastic stenosed tubular segments. Different grades of impairment of the spermatogenesis were observed in this variants of abnormally configurated tubules. This new lesion was found in: 7 crytorchidic testis (5 with ambiguous external genitalia), 3 patients with descended testes that consulted about infertility, 2 patients with testicular microliths and 3 in the testicular biopsy contralateral to the testis excised for testicular cancer. The incidence of abnormal configurated tubules in the different groups of pathologies in our cases was: male psudohermaphroditism 45%, crytorchidism 30%, infertility 15% and testicular contralateral testis 10%.
Conclusion: This study suggests that the abnormal seminiferous tubules distribution may be represented a new histological pattern present in testicular dysgenesis syndrome.

SPERM FUNCTION/SEMEN ANALYSIS

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NYD-SP27, AN INTRINSIC DECAPACITATION FACTOR IN SPERM

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Prior to fertilization sperm have to undergo an activation process known as capaciation, leading to the acrosome reaction, which releases hydrolytic enzymes to facilitate sperm penetration of the egg. However, premature capacitation may lead to untimely release of sperm acrosomal enzymes before they reach the egg. Little is known about the mechanism for preventing premature capacitation in sperm although decapacitation factors from various sources have been thought to be involved.
Objectives: To investigate the role and mechanism of NYD-SP27 in sperm, which is an isoform of phospholipase C (PLC)æ , previously shown to exert an inhibitory effect on the PLC-coupled Ca2+ mobilization in the pancreas.
Methods: We examine the location of NYD-SP27 in sperm by immunofluorescence. CTC and immunofluorescence double staining show that NYD-SP27 is detached from the sperm during capacitation and acrosome reaction. We demonstrate that the specific antibody to NYD-SP27 could block the detachment of NYD-SP27, use the antibody and PLC inhibitor to study the effect on capacitation and acrosome reaction. Also the $[Ca^{2+}]_i$ in sperm is examined to clarify the underlying mechanism.
Results: NYD-SP27 is localized to sperm acrosome and shown to be detached from sperm as they undergo capacitation and acrosome reaction. The absence of HCO3-, a key factor in activating capacitation, from the capacitation-inducing medium prevents the lost of NYD-SP27 from sperm. The antibody against NYD-SP27 also prevents the lost of NYD-SP27 from sperm and significantly reduces the number of capacitated sperm and acrosome reaction induced by ATP and progesterone, as well as inhibits the PLC-coupled Ca2+ mobilization in sperm, which can be mimicked by an inhibitor of PLC, U73122.
Conclusions: These data strongly suggest that NYD-SP27 is a physiological inhibitor of PLC that acts as an intrinsic decapacitation factor in sperm to prevent premature capacitation and acrosome reaction.

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THE SPERM CHROMATIN DISPERSION (SCD) TEST. AN OVERVIEW OF ACTUAL CLINICAL AND BASIC RESEARCH

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Introduction and Objectives: The SCD test, developed as a kit, is a simple and reliable technique to determine sperm cells with fragmented DNA (SFD). We show a general overview of the actual studies and possibilities of this technique.

Methods: The spermatozoa, immersed in a microgel on a slide, are treated with an acid solution and lysed. Sperm nucleoids with small halo size, without halo and without halo and degraded, are those with extensive DNA breaks, whereas those without DNA fragmentation amply release their DNA loops. This was confirmed by sequential DBD-FISH or by enzymatic labelling. SCD can be assessed under bright-field or fluorescence microscopy, being not necessary extra instrumentation The slides may be permanent, and with a control sample.
Results: In 85 couples undergoing IVF/ICSI it was found a negative correlation between SFD and the fertilization rate, synchrony of nucleolar precursor bodies' pattern in pronuclei, ability to achieve blastocyst stage and its morphological quality. Patients with chronic genitourinary infection by *Chlamydia trachomatis* and *Mycoplasma sp.* showed an 3.2 X increase in SFD that reversed after therapy. Patients with varicocele revealed a high proportion of the "degraded" cells in the total of SFD. The SFD were increased in 99 male smokers, after swim-up. Conventional FISH could be sequentially performed on SCD-processed slides, allowing simultaneous determination of aneuploidy and presence of DNA fragmentation. The aneuploidy rate was 4.6 X higher in SFD, so aneuploidy during sperm maturation could lead to sperm DNA fragmentation as part of a screening mechanism to genetically inactivate sperm with a defective genome. This possibility was also derived from studies on knockout mice for telomerase, with critically shortened telomeres. Sequential protein staining showed that SFD also contain a modified nuclear protein matrix. Sequential use of antibodies against 5-methylcytosine, or electron microscopy, may also be accomplished.
Conclusion: Given their peculiarities and possibilities, the SCD kit may be used to easily perform research on human sperm DNA fragmentation, with a high image quality and power of resolution. The possibility of application of other sequential procedures illustrates the great potential and versatility of the kit. This presentation has been supported by ChromaCell SL.

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DNA DAMAGE/CHROMATIN MODIFICATION DURING CRYOPRESERVATION DOES NOT INCREASE IN MORPHOLOGICALLY ABNORMAL SPERM

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Sperm cryopreservation has become an important component of assisted reproduction. During cryopreservation, sperm undergo dramatic changes in both intracellular and extracellular environment due to exposure to cryoprotectives, cooling, freezing, storage in liquid nitrogen, and thawing. The chemical and physical effects of these reagents/processes have a detrimental effect on chromatin, morphology, membrane integrity, and vitality of human spermatozoa.
The present study was undertaken to study the sperm DNA damage and chromatin modification induced during the process of freeze-thawing and to find out its association with sperm morphological abnormality (head abnormality). We used Single-cell gel electrophoresis (SCGE) or comet assay which has the ability to detect damage at the single cell level and acridine orange (AO) binding ability to evaluate the chromatin status of the sperm. In this study, semen samples from 25 males (sperm count >20 millions/ml) attending our center for infertility evaluation were included. After evaluating for sperm count, motility, vitality and morphology (WHO, 1999), the sperm DNA damage and chromatin status were measured by comet assay and AO binding. The semen samples were stored in liquid nitrogen using glycerol egg yolk citrate buffer. After one week, the extent of DNA damage and chromatin status was again evaluated in thawed samples. Although, our study showed a significant increase in the amount of DNA damage and chromatin denaturation ($p<0.05$) after cryopreservation, we did not find any association between sperm morphology and freeze-thaw induced sperm DNA damage. These results demonstrate that the susceptibility of morphologically abnormal sperm to undergo DNA damage/chromatin modification is similar to morphologically normal sperm although cryopreservation process by itself alters the DNA integrity of the sperm.

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FILLING TIME OF A LAMELLAR CAPILLARY FILLING SEMEN ANALYSIS CHAMBER IS A RAPID, PRECISE AND ACCURATE METHOD TO ASSESS VISCOSITY OF SEMINAL PLASMA

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Rapid and easy assessment of the viscosity of seminal plasma and expression of the results in centipoises (cP), the unit of viscosity, was up to now not possible. This means that not much information is available on the association between viscosity and other semen variables and male fertility. In a way, semen viscosity is now a lost variable in the assessment of male fertility. It is presumed that the flow velocity or filling time of capillary filled disposable semen analysis chamber are a measure of the viscosity of a semen sample. The objective of this study was to investigate the use of Leja 2 and 4 chamber disposable semen analysis chambers as viscosity meters
Design of the Study: The viscosities of two hundred and forty eight samples of human seminal plasma were assessed using a viscometer (Vilastic 3). The same samples were used to measure with a stopwatch the filling time of both the Leja 2 and 4 chamber slides. The accuracy of both methods (Vilastic and capillary filled chambers) was assessed with water and culture medium. The precision was assessed with repeated measurements of pooled samples of seminal plasma. The viscosity of 211 (89%) of the samples could be assessed with capillary filled chambers. Thirty seven samples (11%) were too viscous. A linear relation between filling time and viscosity up to viscosities of 8.4 cP was found. The median value of the viscosities of the 211 samples was 4.1 cP and 95% of the viscosities were between 2.8 and 6.8 cP. The coefficient of variation of both methods was 2-5%.
Conclusion: As expected there is a linear relationship between filling time of a capillary and viscosity. The assessments of viscosity with capillary filled disposable chambers is as accurate and precise as the of the viscometer Vilastic 3. The assessment of the filling times takes only 30 seconds to a few minutes. The results are expressed in cP. Our results make viscosity of seminal plasma accessible as a variable to be used in routine semen analysis and allows studies after association between viscosity of seminal plasma and male fertility.

Conflict of Interest: Jan P Vermeiden is director research and development and partial owner of Leja Products BV.

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DETERMINATION OF L-CARNITINE IN HUMAN SEMINAL PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ITS CLINICAL APPLICATION IN MALE INFERTILITY

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Objective: To develop and validate a simple and reliable high performance liquid chromatographic (HPLC) method for the analysis of L-carnitine in human seminal plasma and to investigate its clinical significance as a potentially additional means of evaluating the infertile male.
Methods: After proteins in seminal plasma are precipitated with a mixture of acetonitrile and methanol (9:1; v/v), L-carnitine in seminal plasma was derivatized to form its UV-absorbing ester. HPLC separation of the sample solution was performed on a Lichrospher SiO_2 column and detected by ultraviolet absorbance at 260 nm. A mobile phase composed of acetonitrile-citric acid buffer (containing 12 mmol/L triethanolamine, pH 5.0) was found to be the most suitable for this separation at a flow rate of 1.2 mL/min and enabled the baseline separation of the L-carnitine from interferences with isocratic elution. The L-carnitine levels in seminal plasma were studied in both 30 control subjects and 87 patients with infertility. Ejaculates were classified into studied subgroups and defined as: asthenozoospermia (n=29), oligozoospermia (n=19) and oligoasthenoteratozoospermia (n=39).

Results: Under the chromatographic conditions described, the L-carnitine derivative had a retention time of approximately 13 min. Good separation and detectability of L-carnitine in human seminal plasma sample were obtained. The method proved to be linear in the range of L-carnitine from 0 mmol/L to 1000 mmol/L. The relative standard deviations of within- and between-assay for L-carnitine analysis were 1.23 and 1.36 %, respectively. The recoveries were 91.6 ~ 96.5 % for the human seminal plasma samples. L-carnitine concentrations in the populations were 392.66±107.18 mmol/L in the fertile group (n=30), 270.00±83.92 mmol/L in asthenozoospermia group, 187.97±43.90 mmol/L in oligozoospermia group and 175.65±67.07 mmol/L in oligoasthenozoospermia group. The large difference ($P<0.01$) between the fertile and infertile populations is evident and the difference between the subdivided groups in the infertile group is not significant ($P>0.05$).
Conclusion: The determination of L-carnitine level in seminal plasma may prove useful as a potentially biochemical marker of fertility and this is a useful guidance for the clinic therapy and the mechanismic study on the male reproduction.

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THE SIGNIFICANCE OF DNA OXIDATION IN SPERM QUALITY, SEMEN FREEZING AND CAPACITATION PROCESS A NEW MARKER OF SPERM QUALITY

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Objective: Free radical damage is an important factor in sperm function. Despite extensive efforts there is a great need for additional research on the applicability of free radical damage in humans. One product resulting from free radical damage is the DNA-hydroxylation products known as DNA oxidation. Our aim with this study was to understand oxidative stress effects on DNA damage and determine its role in sperm function by correlating DNA oxidation levels with classic seminal parameters.
Materials and Methods: Semen samples were obtained from infertile males (n = 38) of couples undergoing oocyte donation cycles. All aliquots were examined for ejaculate volume, concentration, motility and morphology. Nineteen samples were previously frozen and thawed with controlled glycerol based method. The other 19 samples were freshly processed. Thawed and fresh samples were capacitated by swim-up method by using Human Tubal Fluid (HTF) media. OXIDNA assay kit was used based on the direct binding of a fluorescent probe to the DNA adduct 8-oxoguanine in damaged cells. Fluorescence from ejaculated and capacitated sperm was quantified using flow cytometry (excitation 495 nm, emission 515 nm). Percentage of stained cells and the average of staining intensity were obtained.
Results: In fresh samples, no significant differences were seen in the percentage of stained cells before after swim-up (40.64% SD=12.6 vs. 43.90% SD=12.5).. After thawing, a significantly higher increase in percentage of cells with oxidized DNA was seen in fresh samples (28.53% SD=11.71 vs 43.98% SD=10.8 p=0.01) meanwhile capacitated samples did not differ from sperm previously thawed or not. Table: Correlation between percentage of cells with DNA damage and sperm parameters before and after swim-up (independently of being fresh or frozen samples).* Denote a linear relationship $p<0.05$.

Parameter	Volume	Concentration	Progr. Motility	Total prog. sperm	Morphology
Fresh	-0.536*	0.124	-0.395*	-0.505*	0.052
Swim-up	-0.168	-0.271	0.018	-0.241	0.109

Conclusion: Oxidative damage in the DNA is clearly increased in frozen-thawed samples; this damage is reflected by a decrease in sperm motility. Nevertheless the DNA damage disappears when the sperm cells are capacitated. Cryopreservation produces an increased oxidative damage in the DNA being this a good biomarker of sperm quality and reflecting the free radical damage in human sperm.

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USING ATOMIC FORCE MICROSCOPY TO STUDY THE ULTRASTRUCTURE'S ALTERATION OF THE RAT SPERM TREATED WITH CHINESE MATERIA MEDICA

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Abstract Objective: To investigate ultrastructural changes, particularly at the surface, that occur in pathological spermatozoa by using atomic force microscopy (AFM) and to examine the morphological alterations responsible for infertile sperm treated with chinese materia medica.
Materials and Methods: Normal fertile and pathological spermatozoa treated with chinese materia medica were examined by using a conventional AFM in a noncontact mode. Sperm of ratwith ligoa-sthenoteratozoospermia (OAT) and asthenozoospermia were obtained from the animal model.
Result(s): Morphological details, topological information, and three-dimensional images of the head, neck, and flagellum are presented for both normal and pathological sperm. The obtained images clearly show dramatic alterations in the morphology of the head, neck, and flagellum of pathological sperm. Even the ultrastructure at the top of the flagellum and the region of the acrosome cap are clearly distinguishable.
Conclusion(s): This study has significant importance not only for identifying spermatozoa alterations but also for understanding morphological defects and their effects on infertility. The whole ultrastructural form of infertility sperm could be improved by Chinese materia medica. By this experiment, AFM should be applied to study the human sperm as an important novel tool.
Key Words: Ultrastructure, infertile sperm, atomic force microscopy, oligo-astenoteratozoospermic(OAT)

FERTILIZATION/GERM CELL DEVELOPMENT/REPRODUCTIVE DEVELOPMENT

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ZONADHESIN-DEFICIENT SPERMATOZOA EXHIBIT DECREASED SPECIES-SPECIFICITY OF ADHESION TO THE ZONA PELLUCIDA

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Zonadhesin is a male germ cell-specific protein that binds in a species-specific mannerto the zona pellucida (ZP) of the oocyte. In mammals, zonadhesin comprises tandem MAM, mucin, and von Willebrand D (VWD) domains. Both the primary structure and the domain stoichiometry of zonadhesin vary drama-tically among species, suggesting a possible biochemical basis for zonadhesin's species-specific binding activity and the relative species-specificity of ZP adhesion. In the mouse, a 20 domain expansion of partial VWD domains increases the protein's size to approximately double that in non-rodents. This expansion ofVWDp domains reflects exon duplication (40 exons from 20 duplicated two-exon cassettes) in the mouse zonadhesin gene (*Zan*). To determine the function of mouse zonadhesin, we assessed the effects on *in vitro* fertilization of domain-specific antibodies to zonadhesin, and characterized the fertility of mice with null *Zan* alleles produced by homologous recombination. *Zan* null males were fertile, and litter sizes were not decreased in comparison to wild type mice. Spermatozoa from null males exhibited no apparent defects in ability to adhere to mouse ZP or to fertilize mouse eggs *in vitro*. Antibodies to the expansion of partial VWD domains bound to a subset of living, capacitated, wild type sperm cells and commensurately blocked adhesion of these cells to the ZP, but did not bind to or block adhesion of zonadhesin null spermatozoa. Remarkably, sperm cells from *Zan* null mice possessed increased ability to adhere to the pig ZP, and this effect was dependent on prior capacitation (4 experiments, 150 total ZP examined per group). Adhesion of spermatozoa from wild type mice to pig ZP was consistently low and independent of capacitation (3.6 ± 1.6 non-capacitated spermatozoa/ZP vs. 5.2 ± 1.9 capacitated spermatozoa/ZP; mean ± SD). In contrast, adhesion of spermatozoa from *Zan* null mice to pig ZP increased three-fold with capacitation (from 5.1 ± 1.9 non-capacitated spermatozoa/ZP to 15.9 ± 3.9 capacitated spermatozoa/ZP), to levels comparable to those for adhesion of pig spermatozoa (14.6 ± 4.6 capacitated spermatozoa/ZP). We conclude that ZP recognition is a degenerate process, involving simultaneous or sequential action of more than one sperm molecule, that includes the interaction of zonadhesin with the ZP after initiation of the acrosome reaction. The results further suggest that the specificity of ZP adhesion is determined at least in part by the species-specific ZP binding activity of zonadhesin.

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L-CARNITINE IMPROVES BLASTOCYST DEVELOPMENT RATE IN MOUSE 2-CELL EMBRYOS

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Introduction and Objectives: Human embryos generated from in vitro fertilization (IVF) exhibit varying degrees of cytoplasmic fragmentation, and abundant evidence demonstrates that cytoplasmic fragmentation in human embryos arises from apoptosis. Apoptosis is accompanied with changes in mitochondrial membrane potential and the release of several death-inducing factors. Mitochondrial membrane potential and energy production in preimplantation embryos have recently been the focus of many studies. L-Carnitine (LC) is able to stabilize the mitochondrial membranes and increase the supply of energy to the organelle (lymphoma cells, fibroblasts and embryonic neurons) and protect the cell from apoptotic death. LC has no major side effects, interactions with other drugs or teratogenicity. The aim of our study was to establish the LC concentration in the mouse embryo culture media that is not embryotoxic and study the effect of LC on the embryogenesis.
Methods: A total of 420 2-cell mouse embryos (Embryotech Laboratories, Inc., Wilmington, MA) were incubated in 7 groups: group 1: control (HTF media only); groups 2-7: varying concentrations of LC (0.3, 0.6, 1.25, 2.5, 5 and 10 mg/mL). LC concentrations were based on previous studies using LC in tissue culture media such as, lymphoma cells, fibroblasts and embryonic neurons. Embryos were incubated at 37°C in 5%CO_2, and assessment of embryos development was done after 72 hours by examining the Blastocyst Development Rate percent (%BDR).
Results: Significant improvement in BDR% was seen at LC 0.3 mg/mL compared with the control (%BDR: 100% vs. 83.3%; P = 0.006). No significant difference in %BDR of LC 0.6mg/mL and the control (%BDR: 81.6 versus 83.3%). Significant decrease in % BDR was seen at 1.25, 2.5 and 5 mg/ml concentration of LC (%BDR: 68.3%, 70%, 71.6% versus control 83.3%). However LC at 10 mg/mL was embryotoxic (BDR%: 35 % vs. 83.4%) (P< 0.001).
Conclusions: LC has dose dependent effect on mouse embryo development. Improvement of %BDR at lower LC concentrations (0.3mg/mL) may be beneficial and may offer a novel approach to improve the embryogenesis in IVF.

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EFFECT OF POLYPEPTIDE BACKBONE FROM ZP3 ON SPERM-ZONA BINDING.

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For mammalian, fertilization begins with species-specific recognition between sperm and egg, which depends upon egg zona pellucida glycoprotein and putative sperm interacting protein(s). In mouse, zona pellucida glycoprotein ZP3 is believed to be the primary receptor for sperm and inducer of sperm acrosomal reaction for a long time, and its function is attributed to the specific O-linked oligosaccharides attached to polypeptide backbone. While lots of reports are focused on mZP3's oligosaccharides in fertilization, there are few concerning the role of polypeptide backbone of mZP3. To investigate whether mZP3 polypeptide backbone is involved in sperm-egg recognition, three partially overlapping cDNA fragments, together covering the whole region of mature mZP3, were cloned into pET28a vector and three recombinant polyHis-tagged proteins were expressed as inclusion bodies in Escherichia coli. After purified under denaturing condition, all three proteins were renatured and inspected by circular dichroism spectrum for conformation. Despite that all three proteins possess a-helices and b-sheets which mean native conformation, only one derived from carboxy terminal show inhibitory effect to the sperm-zona binding during in vitro fertilization. This phenomenon could not be explicated by enhanced arosomal exocytosis rate, in that the acrosomal reaction assay for these two proteins indicated its inability to induce the acrosomal reaction. Our results suggest that the polypeptide backbone of mZP3 interacts with sperm and such interaction plays a significant role in sperm-zona binding event, ultimately the successful fertilization.

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SUBCELLULAR IMMUNOLOCALIZATION OF MONOCLONAL ANTISPERM ANTIBODIES IN HUMAN SPERM CELLS

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Previous investigations in this laboratory generated a collection of monoclonal antibodies (mAbs) directed against superficial antigens of human sperm cells (Tang & Bean, J Androl, 1998). More recent preliminary results suggest that treatment of sperm with some of these antibodies can alter the expected pathway toward acrosomal exocytosis. These observations raise the possibility that those target antigens may have important functions in events preceding fertilization. Here we report the subcellular distribution of binding sites for two antisperm mAbs in Percoll® washed, capacitated, and acrosome induced cell populations. Human sperm cells were dried onto slides, fixed with 100% methanol and incubated with primary, mouse anti-human monoclonal antibodies 77 or 37. Following primary antibody, slides were washed and incubated with RITC-conjugated goat anti-mouse IgG secondary antibody. Staining patterns were evaluated using a Zeiss LSM 510 confocal microscope. mAb 77 revealed the brightest localization signal in Percoll® washed and capacitated cells, both showing diffuse head staining. Immunolocalization of acrosome induced cells with mAb 77 showed no head staining. mAb 37 showed minimal staining of Percoll® washed cells. Following capacitation, whole head staining was apparent. Acrosome induced cells had the brightest mAb 37 signal localized in the posterior head region. These observations confirm that these antibodies recognize different antigens of the sperm cell, both of which experience changes in their location as sperm capacitate and undergo acrosomal exocytosis.

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ROBOTIC VASOVASOSTOMY: INITIAL EXPERIENCE

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Introduction: Robot-assisted operations are gaining popularity in urological procedures. Microsurgical vasovasostomy is at the present time considered to be the most effective procedure for anastomosis of vas deferens after vasectomy. Microsurgical operations can be difficult and should be used only by surgeons who perform the operations frequently. We are reporting our initial experience in robot-assisted vasovasostomy.
Patients and Methods: A patient aged 40 years requested vasovasostomy 5 years after vasectomy. The patient underwent robot-assisted vasovasostomy on the left side and microscopic vasovasostomy on the right side after the patient was consulted in detail regarding the experimental robotic procedure. The procedures were performed through bilateral high vertical scrotal incisions. The ends of vasa were identified, insolated and prepared with patent lumen well exposed. A Microspike approximating clamp was used to stabilize the ends of the vasa. The Da Vinci robot or an operating microscope was then brought in and placed above the surgical field. The anastomosis was performed with 6 mucosal sutures (9-0 nylon) and 4 to 6 muscular sutures (8-0 nylon) under either microscope or with robot assistance. The surgical time and the difficulties of each step of the procedure were recorded. The surgical and post-operative complications as well as recovery time were analyzed.
Results: The operative times were 90 minutes for robotic anastomosis and 60 minutes for microscopic vasovasostomy. No intra-operative or post-operative complications were identified. Hand tremor was noticed during microscopic surgery, but this was not seen in the robotic procedure. Tying the 9-0 and 8-0 sutures was difficult with the robot due to the lack of tactile sensation. These very fine sutures were easily broken with robot. The patient resumed daily activities the same day of the surgery and full activities in two weeks. No follow up semen analysis is available at this time.
Conclusions: Robot-assisted vasovasostomy can be safely performed with advantage of overcoming hand tremor. However, it requires a learning curve to handle the fine sutures. The cost-effectiveness and the patency rate of vasal anastomosis need to be studied in a larger population.

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EFFECT OF COWHAGE (*Mucuna pureins*) ON REPRODUCTION IN MALE ALBINO RATS

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Introduction & Objective: Effect of alcoholic seed extract of cowhage (*Mucuna pruriens*) for aphrodisiac and spermatogenic potential in male albino rats.
Methods: Rats weighing around 180 to 200 gms were divided into, group I (G1) (received saline) and group II (G2), group III (G3) & group IV (G4) received 150, 200 and 250 mg/kg of body weight of extract (seed) respectively. Animals received daily oral feeding of either extract or saline for 50 days. Following investigations were done on 15th, 30th and 50th days of survival Mating behavior: Parameters analyzed were mount latency, intromission latency, ejaculation latency, post-ejaculatory interval, mounting and intromission frequency and Biochemical & Hormonal analysis: Periodical evaluation of blood samples. By 60th day, animals were sacrificed by over dose of anesthesia. Sperm analysis: Epididymal sperm concentration, viability and motility were analyzed. DNA/ Chromatin integrity was studied using Acridine orange (AO), aniline blue (AB) and methyline blue (MB) stains. Anti-oxidant, histological & histomorphometrical study: Testis and epididymis were processed for anti-oxidant estimation, H & E stained sections were used histological and histomorphometric analysis.
Result: Increase in sperm concentration was seen in G2, G3, & G4, than G1, with peak was in G3. However, chromatin integrity was varied in drug administrated groups, than control. Mating behavior scoring indicates an increase in ejaculation time and number of intromission, which was more pronounced in G3, than G2 & G4. Testosterone levels were increased by 15th and 30th days in G2, G3 & G4, maintained at a higher level than normal, without progressive increase with dose. Anti-oxidant levels were near normal in G3, whereas slight increase was observed in others (G2, G3 & G4). Histological study revealed increased spermatogenic activity in G2, G3 & G4. No major toxic side effects were observed.
Conclusion: Increase in ejaculation time, number of mounting and sperm concentration signify that seed of this plant is having the potential to increase sexual activity and sperm production. Effects seem to be dose dependant as G3 exhibit excellent result. Slight fluctuation in sperm DNA/chromatin integrity might be due to increased rate of spermiation. Increase in testosterone level signifies the androgenic property of the extract. Testicular and epididymal weight increase supported by histological analysis clearly demonstrate that, the cowhage having the potential to increase sperm production, as well as producing aphrodisiac effect in male rats. However, a long-term study is warranted to analyze its beneficial and toxic side effects of this extract, before testing it on specific infertility condition.

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RANDOMLY SURVEY OF ERECTILE DYSFUNCTION PRELENCE AND ITS CORRELATIVE RISK FACTORS IN TYPE 2 DIABETIC MALE PATIENTS

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It is well known that diabetes millitus(DM) and Erectile dysfunction(ED) are common chronic disease, and ED may be the initial symptome of DM. Now to investigate the prevalence of erectile dysfunction in type 2 diabetic male patients and analyze its risk factors in Weifang district.

Methods A total of 1100 type 2 diabetic adult married male patients cases were randomly selected form the Weifang district hospital clinic documentary, the International Index of Erectile Function-5(IIEF-5) was given to them by one same physician on face to determine the status and severity of ED. At the same time, factors as age, duration, history of drinking and smoking, blood pressure and anti-hypertension drugs were investigated, and the relationship with ED was analyzed.

Results The amount of qualified questionnaire was 904 case (904/1100, 82.18%). According to the total scores of IIEF-5, the prevalence of ED in male type 2 diabetic patients was 67.7% (612/904). Logistic regression analysis showed that age, the duration of diabetes, Hb_{A1C}, systolic blood pressure, positive history of smoking and drinking were independently associated with the occurrence of ED, the values of OR rise to 1.9617, 1.2519, 2.3207, 1.1219, 1.674 5(p<0.05), 4.3671 (p<0.01), along with adding of 5 years in duration, 10 years in age, 2% in Hb_{A1C}, 4 kPa in systolic blood pressure, positive history of smoking and drinking respectively.

Conclusions Age, the duration of diabetes, Hb_{A1C}, systolic blood pressure, positive history of smoking, and positive history of drinking are independently associated with the occurrence of ED in type 2 diabetic male patients. With the increases of age, the duration of diabetes, Hb_{A1C} and systolic blood pressureÿthe incidence of ED increase. Habit of smoking and drinking will increase the risk of ED significantly.

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CRYPTORCHIDSM: PREPUBERTAL ORCHIOPEXY MAY PREVENT TESTICULAR CANCER

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Abstract Introduction and Objective: Current indications for orchidopexy with cryptorchidism are to decrease the risk of infertility and to facilitate testicular self examination. The latter is important for the early detection of testicular cancer, which has been reported to be 4 to 10 times more common in men with a history of cryptorchidism. Although the increased risk for germ-cell cancer in cryptorchid testes is undisputed, it is unclear whether orchidopexy affects the natural history of testis cancer development. We hypothesize that early orchiopexy is protective against subsequent development of testicular germ-cell cancer.

Methods: A systematic review and meta-analysis were conducted as per guidelines published by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. All literature pertaining to cryptorchidism and testicular cancer risk was retrieved by searching MEDLINE (1966-2006), BIOSIS (1969-2006), and The Cochrane Library using cryptorchidism as a core keyword in combination with treatment, orchidopexy, testis, and cancer. For data extraction, exposure was dichotomized to orchidopexy before or after age 10 to 11 years, while outcome was defined as the development of testicular germ-cell cancer. Statistical analysis and summary risk measures were calculated using the random effects model.

Results: Four observational studies met our criteria. Systematic review revealed an increased risk of developing testicular cancer if orchidopexy was delayed until after age 10 years or never performed (OR 2.9-32.0). Meta-analysis confirmed that men in whom orchidopexy was delayed or not performed were nearly 6 times [OR 5.81 (1.75, 19.26)] more likely to develop testicular cancer compared to those in whom it was performed prior to age 10 years

Conclusions: In cryptorchid males, prepubertal orchidopexy decreases the risk of testicular cancer. While preservation of fertility is important, this is an equally or more important indication for early surgical intervention. Further, these findings suggest that the testicular environment, as well as underlying genetics, may play a role in testis cancer carcinogenesis.

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SPERM QUALITY AND CRYOPRESERVATION TOLERANCE IN MEN DIAGNOSED WITH TESTICULAR AND SYSTEMIC MALIGNANCIES – A COMPARATIVE STUDY

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Infertility is a major sequel of cancer and/or its therapy. The present clinical means for preserving the reproductive capacity of men at this risk is sperm cryopreservation. The objective of our study was to compare sperm quality and cryopreservation tolerance in a group of men diagnosed with testicular malignancy and systemic malignancy.

Specimens were collected from 89 cancer patients prior to the initiation of chemo/radiotherapy. Thirty-nine patients had testicular malignancy (carcinoma/seminoma), while 50 patients had systemic malignancy (leukemia/lymphoma). Sperm cryosurvival rate (CSR) was calculated by dividing motility post-thaw by motility pre-freeze X 100. Healthy proven fertile donors were included as controls (n=20).

Sperm concentration was significantly lower in patients with testicular malignancy compared to those with systemic malignancy and donors ($p<0.001$, $p<0.05$, respectively). Motility was significantly lower in patients with testicular and systemic malignancy compared to donors ($p<0.001$). Similarly, CSR was significantly lower in patients with testicular and systemic malignancy compared to donors ($p<0.01$ and $p<0.001$, respectively).
These results highlight the suboptimal quality of spermatozoa in men diagnosed with cancer even prior to the initiation of chemo/radiotherapy. The lower sperm quality in patients with testicular malignancy indicates the negative effects of local genital tumors. Sperm cryopreservation protocols should be improved to compensate for the decrease in cryopreservation-thawing tolerance demonstrated by cancer patients.

Study Group	Specimens (n)	Concentration (Million/mL)	Motility (%)	CSR (%)
Testicular malignancy	93	34.6±4.1	41.8±16.3	46.9±2.0
Systemic malignancy	96	55.6±6.3	45.8±1.7	41.7±1.9
Fertile Donors	200	66.9±1.5	61.3±0.7	54.9±0.9

CSR=cryosurvival rate. Values are expressed as mean ± standard error of mean.

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TREATING CHRONIC PROSTATITIS BY MEDICATED IRRIGATION AND DRAINAGE VIA PROSTATIC URETHRAL CATHETER TECHNIQUE
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Abstract: Author summarized 10 years' clinical experience in treating chronic prostatitis by medicated irrigation and drainage via prostatic urethral catheter technique. This double-balloon and triple-channel catheter is invented by author himself used for prostatic urethral irrigation and drainage. The results of recent studies on pathogenesis of chronic prostatitis were systematically reviewed. and the clinical use of this technique was discussed.. The information of design principle, basic theoretical evidence, clinical therapeutic mechanism, as well as curative effect of this technique was also provided for discussion.

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SALVAGE THERAPY TRIAL FOR ERECTILE DYSFUNCTION USING PHOSPHODIESTERASE TYPE 5 INHIBITORS AND VITAMIN E
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Introduction and Objectives: Vitamin E is a potent lipid-soluble oxygen free radical scavenger and was demonstrated that it enhanced the therapeutic effect of sildenafil using diabetic animal model by De Young et al. (2004; J Androl. 25, 830-836). We report our initial experience of salvage therapy for low responder to PDE5 inhibitor using combined medication of vitamin E.
Methods: A total of 8 patients unable to obtain full response to PDE5 inhibitor among 82 erectile dysfunction (ED) men who visited between January 2004 to May 2006 were included. They receivedá-tocophenol of 300 mg per day dose at least over 1 month after adequate informed consent and completed IIEF-5 questionnaires to assess the drug efficacy after re-intake of PDE5 inhibitors.
Results: Averaged IIEF5 score elevated from 13.8±3.6 to 17 .5±3.4 after á-tocophenol administration. 3/5 of men who were fully recorded could have improved IIEF-5 score including maximum elevation of 9 points by virtue of á-tocophenol. Furthermore, all patients except one experienced favorable subjective change, the majority of which was increased penile rigidity.
Conclusion: The results of this study show the possibility of effect of vitamin E enhancing the efficacy of PDE5 inhibitor, to our knowledge, firstly in clinical trial.

EPIDIDYMIS

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EFFETS OF VASECTOMY ON THE GENE EXPRESSION PATTERN ALONG THE HUMAN EPIDIDYMIS
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The human epididymis is essential for maturation, transport and storage of sperm. The consequences of vasectomy on epididymal functions are poorly documented but, some reports suggest that it can affect expression of some genes along the human epididymis. We previously reported that the expression of two human epididymal proteins P34H and HE1 is affected by vasectomy; obviously, synthesis of other proteins might be affected as well. In order to further document the effects of vasectomy on epididymal gene expression pattern in human, we used Affymetrix GeneChip U133 plus 2, representing more than 47000 transcriptional variants, including 38500 well characterized human genes. These chips were probed with cRNA isolated from caput, corpus and cauda epididymides of three vasectomised donors of 45-55 years of age. Three men of 26-50 years of age, with no medical pathologies that could affect reproductive function, were used as control. Tissues were obtained with the collaboration of our local organ transplantation program. Total RNA was *in vitro* transcribed to produce a biotinyled cRNA target. Scanned images were extracted with GCOS 1.2 (Affymetrix) while expression levels were analysed with the Limma package and GeneSpring v 7.2 Software. Over 20 000 genes were expressed in the three epididymal segments from vasectomised donors. Among these genes, 1424, 421 and 1171 genes were differentially expressed between the caput and corpus, the corpus and cauda, and the caput and cauda segments (p-value< 0.05), respectively. Regional comparison, using cluster analysis, revealed that the corpus and cauda segments of the epididymis are greatly affected by vasectomy. As previously described, we observed that P34H expression is affected by duct occlusion. Furthermore, we observed that vasectomy affects the expression of genes encoding for proteins, such as: Stanniocalcin 1 in the caput; â-defensin 126 (ESP13.2) in the corpus; and HE3á, HE3â and CRISP-1 in the cauda epididymides. The data generated in this investigation will allow us to understand the consequences of vas deferens occlusion on the human epididymis and elucidate its role in sperm maturation. This work was supported by Canadian Institute for Health Research grant to RS.

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GENE EXPRESSION PROFILE OF THE HUMAN EPIDIDYMIS AS A TOOL TO UNDERSTAND THE HUMAN BLOOD-EPIDIDYMAL BARRIER AND SPERM MATURATION

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The luminal environment present along the epididymis is believed to be essential for spermatozoal maturation and protection from the immune system. This environment is created by tight junctions between adjacent principal cells and other epithelial cell types that are responsible for the formation of the blood-epididymal barrier. In the rat, epididymal tight junctional proteins are comprised of occludin, zonula occludens proteins (TJP-1) and claudins (Cldns). In the human, there is no information on the proteins that comprise epididymal tight junctions. Thus, the understanding of the factors implicated in the maintenance of the human blood-epididymal barrier and their regulation represent an essential goal in understanding the spermatozoal maturation. Our objectives were to assess gene expression profiles in the human epididymis and to identify the components of the blood-epididymal barrier. Epididymides from four fertile patients (29-50 years old) undergoing orchidectomy were obtained and subdivided into three segments. cDNA arrays were used to compare gene expression in each of the three segments. The majority of the genes exhibited a ratio of 1.0 suggesting that their expression levels were similar in all epididymal regions. A certain number of genes, however, were differentially expressed by at least a two-fold change. Genes that were expressed included several encoding for adhesion proteins, such as cadherins. Both E- and P-cadherin were expressed at the mRNA level in all three epididymal segments as well as ?- and ?-catenin. These adhering junction proteins are essential not only for cell adhesion but also for the targeting and assembly of tight junctions. Several Cldns were also detected (Cldns -1 to -12, -14 to -19 and -23), and while most were expressed at similar levels along the human epididymis, there were some notable exceptions, such as Cldns-8 and -10. The genes encoding TJP-1, -2 and -3 were also expressed in all three segments. Subsequent RT-PCR using specific primers for each Cldn confirmed the cDNA array data. Immunocytochemistry, using antibodies directed against Cldns-1, -8, and -10 showed not only that the expression pattern but also the localization of these Cldns differed along the epididymis. In conclusion, gene analysis provides valuable information on expression of epididymal genes that may participate in sperm maturation. We have shown that the human tight and adherens junctional proteins, which are implicated in the formation of the rodent blood-epididymal barrier, are expressed throughout the epididymis. Further studies on the regulation of these genes in fertile versus infertile patients will allow us to better understand how these genes may be altered in infertile patients.

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PUTATIVE IDENTIFICATION OF A NOVEL CLUSTER OF Wfdc PROTEINS WITH EPIDIDYMAL EXPRESSION IN RODENTS

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Background: Epididymal maturation of spermatozoa requires the interaction with a large number of luminal secretory proteins. Defensins and WAP-four-disulfide-core domain (Wfdc) proteins are two related families of small cysteine-rich proteins expressed in the epididymis. Recent studies have uncovered a large number of new members in each of these families. We hypothesized that additional novel epididymal secretory proteins involved in sperm maturation can be identified using a bioinformatic search that combined models from both families.
Methods: Hidden Markov Models (HMM) of a subset of defensin sequences were built from the published mammalian gene databases. These were then used to search the genomic sequences of six mammals including rat, mouse and human. The HMMs were then refined by inclusion of additional hits, including those from Wfdc genes, and grouping of similar sequences in newer models. Repeat iterations were done until no new hits were detected.
Results: Validity of the search technique was confirmed by identification of all the known defensins and several Wfdc genes. A novel cluster of Wfdc-like genes was identified on rat chromosome 10 consisting of a single known gene, Extracellular Peptidase Inhibitor (Expi or WDNM1), and 14 putative duplicates. In mice, a homologous cluster was identified at the syntenic area of chr 11. In humans, the closest match to the cluster was an expressed pseudogene on chr 17 followed by the Wfcd cluster on chr 20 that includes Eppin and human-Epididymal Protein 4 (HE4). Because Expi has been primarily studied as a mammary gland differentiation marker, we used RT-PCR to determine the expression pattern of Expi in rat genito-urinary tissues.

Preliminary results confirmed by sequencing revealed it to be expressed in caput, corpus and cauda epididymis but not in kidney or testis. Experiments to characterize the putative novel genes and their expression pattern are currently ongoing.
Conclusion: We report the identification of a putative cluster of Wfdc genes in rodents. In addition, this is the first report to our knowledge of the *epididymal* expression of Extracellular Peptidase Inhibitor, a gene whose study has been hitherto limited to mammary tissues, but that shares homology with a growing number of epididymal secretory proteins.

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IMMORTALISATION OF BOVINE CAPUT EPIDIDYMAL CELLS
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Since the epididymis is a non proliferating organ, epididymal cells have a very short lifespan in culture. The present work aims to produce immortalized epididymal cells as a long lasting tool to study the endocrinology of the epididymis and their interactions with male gametes. Few approaches have been used so far with epididymal cells from the bovine, a species representing a great availability of these tissues due to the size of the epididymis. Primary cultures were first nucleofected with an integrase (phiC31) and either the T antigen or a telomerase. The second strategy was to infect primary cell cultures with a virus able to recognize bovine cells ; two different viruses were used, coated with distinct surface proteins. Infection with a lentivirus turned out to be the more efficient approach. So far, a lentivirus containing the eGFP was used as a visual marker of infection. Future assays will aim to infect epididymal cells with telomerase encapsidated lentivirus to produce immortalized cells.

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ENVIRONMENT/TOXICOLOGY

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SPERMATIC FUNCTION AND BIOLOGICAL PARAMETERS IN MICE EXPOSED TO SIMULATED INTERMITENT HYPOBARIC HYPOXIA
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Biological and reproductive parameters of young (3 months old) male CF1 mice (Mus musculus) were evaluated after intermitent exposure to simulated hypobaric hypoxia in a hypobaric chamber. Intermitent hypoxia is at present the condition of copper mining work in Northern Chile and its effect in reproductive health is not known. The objective is to evaluate biological and reproductive parameters in mice after intermitent exposure to simulated hypobaric hypoxia.
Material and Methods: Experimental group: (HI 108) 8 mice exposed to Intermitent Hypobaric Hypoxia (8 days in Hypobaric chamber simulating 4600 masl) and thereafter 8 days in Normoxia (500 masl-average altitude of Santiago, Chile). The cycles were carried out up to 108 days. The control group n=108 (6 mice) was kept in Normoxia for 108 days. Al the end of the exposures, the following parameters were measured: body weight (BW), weights of testis (TW), cauda epidydimis (CEW), seminal vesicles (SVW), and prostate (PW). Sperm counts were done in testis (TSC), and epidydimis (ESC). Hematocrit (H), red blood cells count (RBC), Mean corpuscular hemoglobin (HB), and number of reticulocytes (RC) were estimated, and the results are given in the Table below.

Parameters/Group	HI 108 (n=5) Mean±Stand. Dev.	N108 (n=6) Mean±Stand. Dev.
BW (gr)	36.56 ± 2.15	36.57 ± 2.23
TW (mg)	199.49 ± 14.54	200.83 ± 15.88
CEW(mg)	31.01 ± 4.87	37.71 ± 6.74
SVW (mg)	278.66 ± 53.56	287.83 ± 99.46
PW (mg)	80.64 ± 12.58	74.46 ± 27.48
TSC(nº Spz/test) x 10^6	4.15 x 10^6 ± 0.47	5.10 x 10^6 ± 1.24
ESC (nº Spz/cauda) x 10^6	2.43 x 106 ± 0.72 *	7.23 x 106 ± 2.76 *
Hematocrito (%)	40.60 ± 12.25	34.78 ± 2.72
RBC (nº/mm3) x 10^6	7.47 ± 2.18	6.86 ± 0.66
HB (picogram/Cells)	14.28 ± 2.52	12.83 ± 0.99
RC (%)	15.92 ± 4.43	18.40 ± 1.58
	(*)P< 0,05	

Conclusions: Intermitent Hypobaric Hypoxia seems not to affect body weight nor the weights of the reproductive organs or the testicular sperm production but the sperm count in the cauda epididymis decreases in hypoxia, as if maturation and sperm transit were altered. Intrestingly enough, hematological values are not different from normoxia in the hypoxic animals; probably because the normoxic periods tend to bring values to normalcy. However, long term effects on the spermatogenic function must be explored in more detail.

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ESTROGENIC EFFECT OF SOYPROTEIN ON SPERMOGRAM AND GONADAL HORMONES OF MALE ALBINO RATS

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Phytoestrogens are plant-derived, non-steroidal compounds that bind to the estrogen receptor and can induce estrogen-dependent gene transcription. Soybean, which is an important source of protein in the diet of many countries in Africa, especially Nigeria, has been found to contain naturally occurring phytoestrogens. We have earlier reported a deleterious effect of soy-protein on reproductive system of female rabbits. In the current study, we investigated the estrogenic effect of phytoestrogen on reproductive system of male albino rat. Eighteen albino rats were divided into three groups consisting of six each. While the phytoestrogen group were fed with soy-protein supplemented feeds, other groups received normal feed. The estrogen group were given subcutaneous injection of estrogen while the other groups received normal physiological saline. The weight and length of the organs were measured using either electronic weighing machine (Mettlar AE 240) or flexible tape as appropriate. Semen samples from the epididymis were analyzed by the conventional methods. Testosterone and estradiol level were evaluated by the enzyme immunoassay (EIA) technique (W.H.O.). Animals injected with estrogen lostweight significantly ($p<0.001$), while those on phytoestrogen gained considerable weight comparable with normal controls. We observed significant decrease in the testicular weight, the circumference of the testis and length of epididymis of the test animals compared with the control ($p< 0.05$). Furthermore, there was a significant reduction in sperm count, motility, viability and morphology, and hence the quality of semen from phytoestrogen and estrogen group. However these differences were more pronounced in the later (estrogen). Although the serum estrogen level increased significantly only in the group on estrogen, the serum testosterone significantly reduced in both phytoestrogen and estrogen groups ($p<0.005$). These results indicate that environmental hormone-like exposure as in dietary soy-protein contributes to decline in fertility of male albino rat. This warrants investigation of possible use of phytoestrogen in fertility control.

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OCTYLPHENOL DOES NOT ALTER MALE REPRODUCTIVE TISSUES, EPIDIDYMAL SPERM MOTILITY, OR TESTICULAR GENE EXPRESSION IN SPRAGUE-DAWLEY RATS

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p-tert-octylphenol (OP) is a degradation product of alkylphenol ethoxylates, commonly used surfactants. OP is known to selectively bind to the estrogen receptor; however, reports of its effects on males are controversial. The objective of this study was to evaluate the effects of OP on various male reproductive parameters. To evaluate short-term effects, adult Sprague-Dawley male rats were gavaged with a high dose (125 mg/kg) of OP, which had been reported previously to cause testicular toxicity. Rats were sacrificed 24 hours later. No significant differences were observed in body or sex accessory gland weights. Analyses with Agilent cDNA arrays revealed that OP treatment did not alter testicular gene expression. To assess long-term exposure of OP on male reproduction, rats were given OP for 60 days. Five groups were used: an untreated control, a vehicle control and three doses of OP (25, 50, and 125 mg/kg bw), administered daily by gavage to male Sprague-Dawley rats. A trend towards decreased body weight, relative to untreated controls, was observed in all OP-treated groups, with a significant decrease in body weight in the 125 mg/kg group. Tissue levels of OP in the testis were below levels of detection in all OP groups except the 125 mg/kg group, where OP levels were 29.3 + 16.1 ng/g of tissue. In the epididymis, OP levels were below detection in the 0 and 25 mg/kg groups, while levels in the 50 mg/kg group were 18.4 + 15.9 ng/g, and in the 125 mg/kg group reached 56.3+ 45.3 ng/g. Both testicular and epididymal histology were not altered by treatment with OP at any of the doses administered. Assessment of sperm motility indicated that total percent motility in the intermediate dose (50 mg/kg bw) was lower (13%) than in untreated controls, and a tendency for an increased % of static cells was also observed in all OP groups, with the intermediate dose (50 mg/kg) displaying a higher % of static cells (28%), relative to untreated controls (15.7%). Sperm counts were also slightly lower in all OP groups, and significantly lower at the highest OP dose (125 mg/kg bw), relative to untreated controls. In conclusion, daily OP exposure via gavage for 60 days, at doses ranging from 25-125 mg/kg bw, did not appear to exert any major detrimental effects on male reproductive organs or motility-associated parameters, and only slightly affected sperm motility and sperm counts. Gene expression profiles of testes indicated that expression of 15 genes was modulated by OP treatment; however, these differences were not statistically significant. Previous studies have reported that OP administration to rats caused gross morphological alterations to the testis and epididymis and that sperm motility was significantly altered. Recent publications have failed to reproduce these earlier observations but suggest effects on the histology of the testis and epididymis. Our results indicate that OP treatment does not exert any major effects on male reproductive function.

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MELATONIN PREVENTS DAMAGE OF MOUDE SPERM DNA ELICITED BY THE ORGANOPHOSPHORATE PESTICIDE DIAZINONÒ

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Toxic effects of pesticides are usually associated of DNA damage. The aim of this work us to evaluate the effect of the organophosphorate pesticide DiazinonÒ (D) on mouse sperm and test if Melatonin inhibits or decrease the damage, which is supposed to be mediated by reactive oxygen species (ROS). Melatonin (M), is known to be a very powerful ROS Scavenger.

Material and Methods: 72 CF1 young adult male mice (30 + 2 g weight, 10-12 week of age) were separated in 12 cages (6 animal each) and kept at 12:12 h light/darkness, temperature 20-22ºC, fed with commercial pellet and water ad-libitum. Controls were intraperatoneally (ip.) injected with 0,2 mL of 0,9 % NaCl (group 1), DiazinonÒ 1/3 LD50 (group 2) or 2/3 LD50 (group 3) Melatonine 10 mg/Kg body weight ip. 30 mi. before D administration (group 4), D1/3 LD50 + M (group 5), D 2/3 LD50 + M (group 6). Cauda epididymal sperm were obtained at day 1 and 32 after injection to analyze chromatin changes.

Results and Discussion: Acridine organize (AO), staining of sperm revealed the first day DNA damage in the mice treated with D 1/3 or 2/3 LD50 (24,3 % and 30,8 % of metachromatic nuclei respectively) rupture of the DNA double helix, a change that was not seen in the mice exposed to D but treated also with M, which are not different from controls. By 32 days there is no damage detected by AO. The comet assay (to detect DNA fragmentation) shows more DNA damage only at day 1 in the group D 2/3 LD50 (34,2%) compared to controls (15,2%). The group of 1 day treated with D showed less packing of the chromatin 1/3 LD50 (81 %) and 2/3 LD50 (63%) when comparing them with the control (88,7). While, the groups of day 32 do not show differences with regard to the controls. Micronuclei test done in red bone marrow cells demonstrates somatic cell chromatin damage in groups treated with D 1/3 (14%) and D2/3 (28%) LD50. In the groups that received M and D, no difference was found to the control value (6%). In conclusion the organophosphate pesticide D is a testicular toxicant that alters sperm DNA structure. M is able to avoid this damage in the conditions tested in this work.

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