35th Annual Meeting

April 10–13, 2010
Houston, Texas

Program and Abstracts
35th ASA Annual Meeting  
April 10 – 13, 2010

Andrology Lab Workshop  
April 10 – 11, 2010

ASA Special Symposium  
April 10, 2010

NEW in 2010: Basic Science Workshop  
April 10, 2010

ASA 35th Annual Meeting  
Lifetime Determinants of Male Reproductive Health  
April 10 – 13, 2010

Program Chairs: Kate Loveland, PhD and Mark Sigman, MD

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

*Please note that all sessions will be held in Regency Ballroom DEFG unless otherwise noted.

FRIDAY, APRIL 9, 2010

2:00 p.m. – 6:00 p.m.  
Registration  
Location: Regency Ballroom Foyer

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

4:00 p.m. – 9:30 p.m.  
Exhibit Hall Open  
Location: Colonnade Salon

6:00 p.m. – 6:10 p.m.  
Welcome and Opening Remarks

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

6:30 p.m. – 7:30 p.m.  
EMIL STEINBERGER MEMORIAL LECTURE  
Insights into Disorders of Testis Development Using Whole Genome Analysis  
Andrew Sinclair, PhD  
Murdoch Children’s Research Institute, Australia  
(Introduced by Dolores Lamb, PhD)

7:30 p.m. – 7:50 p.m.  
Updates from NICHD & NIEHS  
Stuart B. Moss, PhD  
NICHD  
Jerry J. Heindel, PhD  
NIEHS

7:50 p.m. – 9:30 p.m.  
Welcome Reception  
Location: Colonnade Salon

SUNDAY, APRIL 11, 2010

6:30 a.m. – 8:00 a.m.  
Past President’s Breakfast  
Location: Woodway

7:00 a.m. – 4:00 p.m.  
Exhibit Hall Open  
Location: Colonnade Salon

7:00 a.m. – 6:00 p.m.  
Registration  
Location: Regency Ballroom Foyer

*Schedules subject to change

8:00 a.m. – 9:00 a.m.  
AUA LECTURE  
Melding Basic Research With Clinical Infertility Needs — A Glass Half Empty or Half Full  
Larry I. Lipshultz, MD  
Baylor College of Medicine  
(Introduced by Mark Sigman, MD)

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

9:15 a.m. – 10:45 a.m.  
SYMPOSIUM I — Life in the Womb: Fetal Determinants of Men’s Reproductive Health  
Co-Chairs: John H. Richburg, PhD  
Robert D. Oates, MD

10:45 a.m. – 11:00 a.m.  
Break – Visit Exhibits  
Location: Colonnade Salon

11:00 a.m. – 12:30 p.m.  
Poster Session I  
Location: Colonnade Salon

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: Windsor

“From Mentee to Mentoring: Preparing Women for Success in Science Careers”  
Gayle Slaughter, PhD  
Baylor College of Medicine

CONCURRENT ORAL SESSIONS

ORAL SESSION I — BASIC SCIENCE  
Location: Regency Ballroom B  
Moderators: Ina Dobrinski, DVM, PhD  
Greg Bucho/D, PhD

2:00 P.M. – 3:30 P.M.

ORAL SESSION II — CLINICAL  
Location: Regency Ballroom C  
Moderators: John K. Amory, MD  
Shayne Louis, MD

2:00 P.M. – 3:30 P.M.

Refreshment Break  
Location: Colonnade Salon

LECTURE I  
Erectile Dysfunction: A Red Flag for Early Cardiometabolic Dysfunction  
Martin Miner, MD  
Brown University  
(Introduced by J. Lisa Tenover, MD, PhD)

4:00 p.m. – 4:45 p.m.

LECTURE II  
The Progression to Androgen-Independent Prostate Cancer  
Robert J. Matusik, PhD  
Vanderbilt University  
(Introduced by Gail S. Prins, PhD)

4:45 p.m. – 5:30 p.m.
MONDAY, APRIL 12, 2010

7:00 a.m. – 4:00 p.m.
Exhibit Hall Open
Location: Colonnade Salon

7:00 a.m. – 6:00 p.m.
Registration
Location: Regency Ballroom Foyer

8:00 a.m. – 9:00 a.m.
**WOMEN IN ANDROLOGY LECTURE**
Epigenetic Changes in Sperm: A Cause of Male Factor Infertility
Rebecca Z. Sokol, MD, MPH
University of Southern California
(Introduced by Moira K. O'Bryan, BSc, PhD)

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

9:15 a.m. – 10:45 a.m.
**SYMPOSIUM II – Size Does Matter!**
Growth in the Juvenile
Co-Chairs: Martin M. Matzuk, MD, PhD
Catherine Itman, PhD

10:45 a.m. – 11:00 a.m.
Break – Visit Exhibits
Location: Colonnade Salon

11:00 a.m. – 12:30 p.m.
Poster Session II
Location: Colonnade Salon

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 AND TRAINEE AFFAIRS COMMITTEES
(Not included in registration; tickets required)
Location: Regency Ballroom C

"So You Want to Write a Grant Application to the NIH: How the Program Officer and Scientific Review Officer Can Help"
Stuart B. Moss, PhD
NICH

2:00 p.m. – 3:30 p.m.
**SYMPOSIUM III – Fertility in Adult Life**
Co-Chairs: Jeffrey J. Lysiak, PhD
Cigdem Tanrikut, MD

Roles of Testicular MicroRNAs
Wei Yan, MD, PhD
University of Nevada

Sperm and Your Natural Marijuana: Endocannabinoids, Nicotine and Fertility
Lani Burkman, PhD
University of Buffalo

Phosphorylation Events During Sperm Capacitation
Pablo E. Visconti, PhD
University of Massachusetts

3:30 p.m. – 4:00 p.m.

4:00 p.m. – 4:45 p.m.

4:45 p.m. – 5:30 p.m.

5:30 p.m.

7:00 p.m. – 11:00 p.m.
Annual Banquet
(Not included in registration fee; tickets required)
Location: Black Swan Nightclub
(on property)

TUESDAY, APRIL 13, 2010

7:00 a.m. – 8:00 a.m.
2010 Program Committee Meeting
Location: Westbury

7:30 a.m. – 12:15 p.m.
Registration
Location: Regency Ballroom Foyer

8:00 a.m. – 9:30 a.m.
**SYMPOSIUM IV – Aging and Male Reproductive Health**
Co-Chairs: Kirk C. Lo, MD, FRCS C
Douglas Stocco, PhD

Effects of Aging and Environmental Insults on the Sperm Genome
Andrew J. Wyrobek, PhD
Lawrence Berkeley Labs

Testosterone and the Aging Male
Alvin M. Matsumoto, MD
University of Washington

The Relationship of Paternal Age to Health and Disease in Offspring
Susan Harlap, MD
New York University
9:30 a.m. – 9:45 a.m.  Break – Visit Exhibits
Location: Colonnade Salon

9:45 a.m. – 10:45 a.m.  INTERNATIONAL LECTURE:
Mechanisms of Fertilization –
A View Through Gene Manipulated Mice
Masaru Okabe, PhD
Osaka University
(Introduced by Patricia S. Cutasnicu, PhD)

10:45 a.m. – 12:15 p.m.  SYMPOSIUM V – Long-Lasting Sex
Co-Chairs:  Marvin L. Meistrich, PhD
            Ajay K. Nangia, MBBS

The Therapeutic Potential of Germ Stem Cells
Amander Clark, PhD
University of California, Los Angeles

Love and Monogamy:
An Endocrine Story
C. Sue Carter, PhD
University of Illinois at Chicago

Long-Lasting S-E-X
(Science, Education, Xenogamy)
Anna Steinberger, PhD
Professor Emerita, UTMS-Houston

MEETING ADJOURNED

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schedule at a glance

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I am delighted to invite you to the 34th annual meeting of the American Society of Andrology (ASA) to be held at the Omni Houston Hotel in Houston, Texas (April 10 – 13, 2010). Houston is at its best in the springtime when the weather is spectacular and the azaleas are in bloom. While Houston is known to be the home to the largest medical center in the World, the Texas Medical Center, we are also frequently thought of as the home of the NASA manned space flight program. Importantly, Houston is a city to visit, dine in and enjoy. The meeting will be held in the Galleria area close to shopping, ice-skating and restaurants that range from world-class barbeque from TexMex to Vietnamese/Asian to fine dining.

Our distinguished and worthy program co-chairs, Kate Loveland and Mark Sigman (together with the Program Committee), have constructed a broadly focused and scientifically stimulating meeting aptly entitled "Lifetime Determinants of Male Reproductive Health". The program includes a number of renowned keynote speakers who will deliver lectures and host symposia and related didactic sessions. At this meeting the first Emil Steinberger, MD Memorial Lecture will be held to honor one of the founding fathers and first President of the ASA. Full details of the program highlights can be accessed at the ASA website www.andrologysociety.org.

Drs. Run Wang and Robert Brannigan will again host the popular ASA Special Symposium on Saturday afternoon entitled "Challenges in Urological Andrology". This session will focus on major clinical and surgical themes in andrology. The Andrology Lab Workshop, one of the most popular sessions of the meeting, chaired by Dean Morbeck, is now held over 1½ days (all day Saturday and Sunday morning, April 10 – 11). A new session, "The Basic Science Workshop will be held on April 10, 2010. This session, directed by Kate Loveland, will be open to trainees wanting to learn state-of-the-art methods in andrology research.

Marvin Meistrich and I, your local hosts, have arranged a festive welcome reception and annual banquet and we are certain that both venues will offer a glimpse of Houston hospitality and a wonderful time for all attendees.

Dolores J. Lamb, PhD, HCLD
President, ASA

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Past Presidents of the American Society of Andrology

1992 - 1993  Ronald S. Swerdloff  *Deceased
1993 - 1994  Bernard Robaire

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*Deceased
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Secretary Janice L. Bailey, PhD
Treasurer Erwin Goldberg, PhD
Past President Wayne J.G. Hellstrom, MD

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International Liaison Committee Patricia S. Cuasnicu, PhD; Buenos Aires, Argentina
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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.
Overview
The magnificent city of Houston, Texas is home to a thriving economy, breathtaking surroundings, and an inviting community. Houston is a popular international destination. Downtown Houston is especially booming after its recent renewal, resulting in a fresh urban atmosphere with endless dining and entertainment options.

Weather
April is one of the most beautiful times of the year in Houston, Texas with an average high of 79°F and an average low of 57°F.

Shopping
Since shopping has often been called the unofficial pastime of many Houstonians, the city is home to enough stores and boutiques to satisfy any shopaholic. In Houston you can find anything from designer boutiques and upscale specialty stores to flea markets. The Galleria is legendary for its variety of shops and international ambiance.

Art & Theater
Houston is one of the few American cities with companies in theater, ballet, symphony and opera with wonderful performances all year long. Located downtown, the 17-block Theater District is home to eight performing arts organizations. The Houston Museum District is one of the largest museum campuses in the country. It includes 18 institutions within walking distance of one another. Newcomers are always invited to enjoy an exciting symphony or graceful ballet performance in Houston.

Attractions
The Houston Space Center, Downtown Aquarium and Houston Zoo are just a few of the most-visited attractions in the city. The Lynchburg Ferry and Market Square Historic District will appeal to history buffs. The scenic Memorial Park offers six miles of recreation trails along the bayou, while sports fans can enjoy Minute Maid Park as well as the recently constructed Toyota Center.

Dining
The menus of Houston offer virtually unlimited dining possibilities: Creole favorites, fresh-from-the-Gulf seafood, Tex-Mex, and unlimited ethnic options. In fact, because of Houston's diverse population and moderate climate Houstonians dine out more frequently than residents of any other major US city. Perhaps it's that mixture of Southern propriety, Texas hospitality and outstanding cuisine that makes Houston's food so celebrated.

Hotel Information
Omni Houston Hotel
4 Riverway
Houston, TX 77056
Phone: (713) 871-8181
Fax: (713) 871-0719
Website: www.omnihotels.com

Hotel Accommodations
$169.00 per night plus applicable state and local taxes, fees and assessments (currently 17%, subject to change). Reservations can be made by calling the Omni Reservations Department at 800-THE-OMNI (800-843-6664) and requesting the "American Society of Andrology 2010 Annual Meeting" group rate. All reservations must be guaranteed with a valid major credit card. Any guaranteed reservation not cancelled 24 hours prior to arrival will be subject to a one-night room and tax cancellation fee. Note that check-in is at 3:00 p.m. and checkout is at 12:00 p.m. Guests will need to provide a form of payment (credit card) to guarantee the reservation at the time of making the reservation.

Transportation

Airport Information
George Bush Intercontinental Airport (approximately 30 minutes)
Directions to Hotel: I-45 South to Loop 610 West (becomes 610 South); exit Woodway and make a right. Proceed west for approximately two blocks. Turn left on Riverway, indicated by two large red walls on your left hand side. Continue on Riverway until you reach the hotel.

William P. Hobby Airport (approximately 25 minutes)
Directions to Hotel: I-45 North to Loop 610 West (becomes 610 North); exit Memorial/Woodway. Turn left at Woodway and proceed two blocks. Turn left on Riverway, indicated by large red wall.

Car Rental
Avis Rent-A-Car is the official rental car company for the ASA 2010 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 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.

Taxi Service
George Bush Intercontinental Airport
Taxis can be hailed through the Ground Transportation employees outside each terminal.
Yellow Cab: (713) 224-4445
Rates: Approximately $55.00 (one way)

William P. Hobby Airport
Taxis are available at Curb Zone 3 outside of the baggage claim area in the lower level.
Yellow Cab: (713) 224-4445
Rates: Approximately $49.00 (one way)

Shuttle Service
SuperShuttle provides twenty-four hour ground transportation services at George Bush Intercontinental and William P. Hobby airports on a time-scheduled basis. Reservations are encouraged to and from the airport. You can reach Super Shuttle at (800) 258-3826 or online at www.supershuttle.com.
Rates: Approximately $25.00 (one way per person)
Laboratory Science Forum Luncheon
“Sperm Chromatin Structure: To Protect and Release”
Date: Saturday, April 10, 2010
Time: 11:45 a.m. – 1:15 p.m.
Location: Regency BC
Dr. Björndahl will present an overview of the sequence of sperm chromatin packaging during spermatocytogenesis, spermiogenesis, after spermiation, during epididymal transit, after ejaculation, and after laboratory processing of semen. Included will be a summary of the different tests of chromatin structure and the relevance of each.
Cost: $35.00 for non-ALW registrations. Pre-registration required.

Welcome Reception
Date: Saturday, April 10, 2010
Time: 7:50 p.m. – 9:30 p.m.
Location: Colonnade Salon
Join us for a welcome reception to connect with friends and colleagues. Admission to the reception is included in your ASA registration fee; however, it is not included if you are only attending the Basic Science Workshop, Special Symposium and/or Andrology Lab Workshop.
Dress: Business casual or casual attire is appropriate

Women in Andrology Luncheon and Discussion
“From Mentee to Mentoring: Preparing Women for Success in Science Careers”
Date: Sunday, April 11, 2010
Time: 12:30 p.m. – 2:00 p.m.
Location: Windsor
Gayle Slaugher, PhD, is a professor in the Department of Molecular and Cellular Biology, as well as assistant dean for the Graduate School of Biomedical Sciences at Baylor College of Medicine in Houston. There, she creates and directs a series of programs to identify and nurture young scientists from college students to post-docs. She also developed and presented a range of skills workshops to help scientists learn how to optimize their results and time. In this session, Dr. Slaugher will discuss different types of mentors throughout a career and provide pointers on being mentored and being a mentor, including results from a survey of undergraduate women regarding characteristics of valuable mentors.
Cost: $25.00 per person. Pre-registration required.

Trainee Forum and Mixer
Date: Sunday, April 11, 2010
Time: 6:30 p.m. – 8:30 p.m.
Location: Regency Ballroom ABC
The ASA Trainee Forum and Mixer provides 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. Pre-registration required.

Mentoring Luncheon sponsored by the Diversity and Trainee Affairs Committee
“So You Want to Write a Grant Application to the NIH: How the Program Officer and Scientific Review Officer Can Help”
Date: Monday, April 12, 2010
Location: Regency Ballroom C
Time: 12:30 p.m. – 2:00 p.m.
“I just pressed 'submit' to send my application (and my professional life) electronically to the NIH. Where does it go and what happens next?" The NIH are mainly involved in the review process: the Program Officer and the Scientific Review Officer. Each has a distinct and essentially non-overlapping job, and it is important to know who to call when, and what information each may be able to provide. We will discuss the different funding mechanisms (e.g., R01, R21, R03) and how to try to target your proposal to the most appropriate study section for review. Finally, we will discuss funding opportunities for the male reproductive health field.
Speaker: Stuart B. Moss, PhD, Program Director for Male Reproductive Health, Reproductive Sciences Branch, The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health
Cost: $5.00 for trainees, $10.00 for non-trainees. Pre-registration required.

Annual Banquet
Date: Monday, April 12, 2010
Time: 7:00 p.m. – 11:00 p.m.
Location: Black Swan Nightclub (on property)
Enjoy an evening of dinner and fun with colleagues at the famed Black Swan at the Omni Houston Hotel, one of Houston's premier nightclubs. The Black Swan is your direct access to excitement and entertainment, as well as the place to see and be seen.
Cost: $75.00 per person, $35.00 for trainees. Includes dinner and entertainment. Pre-registration required.
Dress: Business Casual

PROGRAM COMMITTEE
Kate Loveland, PhD; Clayton, Victoria, Australia (Co-Chair)
Mark Sigman, MD; Providence, RI (Co-Chair)
John K. Amory, MD; Seattle, WA
Janice L. Bailey, PhD; Quebec, QC, Canada
Patrick S. Cuasnicu, PhD; Buenos Aires, Argentina
Ina Dobrinski, DVM, PhD; Calgary, AB, Canada
Barry T. Hinton, PhD; Charlottesville, VA
Peter Nicholas Kolettis, MD; Birmingham, AL
Dolores Lamb, PhD; Houston, TX
Martin M. Matzuk, MD, PhD; Houston, TX
Robert D. Oates, MD; Boston, MA
Michael A. Palladino, PhD; West Long Branch, NJ
Gail S. Prins, PhD; Chicago, IL
Tracy L. Rankin; Bethesda, MD
message from the program co-chairs

On behalf of the 2010 Program Committee we welcome you to Houston and the 35th Annual Meeting of the American Society for Andrology (ASA).

Research in the biomedical sciences has been transformed by the discovery that events during fetal and juvenile life are critical determinants of adult health. Contributions from the fields of andrology and reproductive biology have led this revolution. To capitalize on this wealth of new knowledge the theme of this year's ASA meeting is "Lifetime Determinants of Male Reproductive Health." The program offers something for everyone with lectures covering all male reproductive organs. Presentations extend from molecular and cellular biology to applications in clinical medicine and will be of interest to basic scientists and clinicians. The program progresses from the prenatal period through development, adulthood, and senescence.

The meeting will open with the keynote talk, the first Emil Steinberger Memorial Lecture, presented by Andrew Sinclair from the Murdoch Children's Research Institute in Melbourne, Australia. Discoveries over the span of his distinguished research career have identified the key genetic switches that are essential for normal sexual development and his presentation will provide an up-to-date insight into disorders of testes development using whole genome analysis.

We are delighted to offer ASA delegates the opportunity to learn from plenary talks by clinical and basic experts each of whom are each making innovative contributions to our understanding of male reproductive health in key areas of our discipline. The American Urological Association Lecture in 2010 is presented by Larry Lipshultz from Baylor College of Medicine. Entitled "Melding Basic Research with Clinical Infertility Needs -- A Glass Half Empty or Half Full?", this talk will point out areas in clinical male infertility that could benefit from additional basic science research. The 2010 Women in Andrology Lecture by Rebecca Sokol from the University of California, Los Angeles, will describe discoveries showing that epigenetic changes in sperm contribute to male factor infertility, an area of research that is emerging as highly important for clinical outcomes. Our ASA International Lecturer is Masuro Okabe from Osaka University in Japan. His contributions to understanding the mechanisms underpinning fertilization are based on intriguing approaches which include the development and application of genetically modified mouse models. Martin Minor from Brown University will present evidence linking erectile dysfunction with cardiovascular disease and metabolic syndrome in a talk entitled "Erectile Dysfunction: A Red Flag for Early Cardiometabolic Dysfunction". Robert Matusik from Vanderbilt University will discuss his group's latest advances in understanding the mechanisms by which progression to androgen independent prostate cancer occurs, and the expert on male reproductive tract function, Bernard Robaire from McGill University, will present his group's latest research. Christina Wang from UCLA Medical Center will share her insights based on a career that bridges basic and clinical research activities, discussing the translation of basic science to clinical andrology with data from her Klinefelter mouse models.

In addition to the plenary talks there will be five symposia representing five progressive phases of male reproductive development and disease, with each set of talks highlighting current developments by international experts. Symposium 1, entitled "Life in the Womb: Fetal Determinants of Men's Reproductive Health", will include three talks discussing the relationship to fertility in adulthood of germ cell epigenetic reprogramming, animal models of testicular cancer, and human testis development. Symposium 2 is entitled "Does Size Matter?: Growth in the Juvenile." Presentations in this session will discuss the importance of Doublesex-Related genes in somatic and germ cell development; the role of vitamin A in controlling meiosis, and the regulation of normal and abnormal pubertal patterns. Symposium 3 talks will focus on fertility in adulthood, with topics: the contribution of microRNAs to spermatogenesis, the effects of obesity and cannabinoids on fertility, and the role of phosphorylation events during sperm capacitation. Symposium 4 will address aging and male reproductive health with discussions on the effects of aging and environmental insults on the sperm genome, the relationship between testosterone and the aging male, as well as the relationship of paternal age to health and disease in offspring. The final symposium, entitled "Long Lasting Sex", will discuss the therapeutic potential of germ cells and the relationship between monogamy and the endocrine system. This session will end with the presentation by Dr. Anna Steinberger, Professor of Emeritus at UTMS Houston, of "Long Lasting SEX (Science, Education, Xenogamy)".

The program was designed to help basic scientists and clinicians to define common areas of interest that will lead to new research opportunities and improved clinical outcomes. We hope that the series of lectures will enable ASA delegates to explore new links between emerging technologies and clinical problems. The members of this year's Program Committee deserve a great deal of credit for the selection of speakers for this annual meeting. The members include Janice Bailey, PhD, Barry Hinton, PhD, John Amory, MD, Gail Prins, PhD, Robert Oates, MD, Patricia Cusznick, PhD, Dolores Lamb, PhD, Michael Palladino, PhD, Ina Dobrinkski, DVM, PhD, Peter Koletti, MD, and Tracy Rankin.

A regular feature of the annual meeting includes the ASAAndrology Laboratory Workshop and Laboratory Science Forum, which will be held on April 10th and 11th, beginning before the annual meeting. The 2010 Andrology Laboratory Workshop program is entitled "Sperm Morphology: A Hands-On Workshop" to be chaired by Dean Morbeck, PhD, HCLD and features members Aniela Bollendorf, MT, David Karabinus, PhD, HCLD, Steve Simon, PhD, Angela Reese, TS, Susan Rothmann, PhD, HCLD, Steven Schrader, PhD and Steve Simon, PhD. The Laboratory Science Forum Luncheon speaker, Lars Bjordalh, MD, PhD, from Karolinska University Hospital, will discuss Sperm Chromatin Structure.

A special ASA Basic Science Workshop will also run during the day before the start of the annual meeting on April 10th. Designed to provide an interactive forum for PhD students, postdocs, research assistants and lab heads, the workshop will be organized by Kate Loveland, PhD and session convenors Sarah Meachem, PhD, Marie-Claude Hofmann, PhD, and Moira O'Bryan, PhD. Topics to be addressed include "Cell Type Identification Strategies", "Cell Culture and Separation Approaches" and "Function/ Fertility Assessments". Contributions from all delegates engaged in basic research are encouraged to make this a valuable opportunity for networking, problem-solving and building collaborations within the ASA.

A special ASA Clinical Symposium, "Challenges in Urological Andrology" will also run before the start of the annual meeting on April 10th, chaired by Robert Brannigan, MD and Run Wang, MD. This will feature presentations by Wayne Hellstrom, PhD on premature ejaculation, John Mulhall, MD on erectile dysfunction therapies, Peyronie's disease by Mohit Khera, MD, management of hypogonadism by Larry Lipshultz, MD, sperm transport and capacitation by Erma Drobnis, PhD, vasectomy by Jay Sandlow, and penile surgical procedures by Rafael Carrion, MD, Gerard Henry, MD and Zhongcheng Xin, MD.

In addition we wish to acknowledge the continuing support of the meeting by the Eunice Kennedy Shriver 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 in this program book, and ASA members. We are grateful to all of the sponsors for their ongoing and generous support of our society. Finally, we thank all those who work in the ASA Executive Office at WJ Weiser and Associates, especially Debbie Roller, Ruth Gottmann, and Marc Cakanic for their assistance in all aspects of creating and executing this meeting.

Sincerely,
Kate Loveland
Mark Sigman
Andrew Sinclair is a professor in the Department of Pediatrics at the University of Melbourne and a director of the Early Development and Disease Division at the Murdoch Children's Research Institute in Melbourne, Australia. His research focuses on understanding the molecular genetics of gonad development and how this impacts on human disorders of sex development. His contributions have been fundamental to the advancement of the field, including the identification and characterization of the human testis determining gene (SRY) and other genes critical for gonad development. Professor Sinclair has an extensive publication record, including seven Nature papers, with one listed as an ISI citation classic. Professor Sinclair leads a National Health and Medical Research Council (NHMRC) Program Grant on human disorders of sex development and is part of a team awarded an Australian Research Council (ARC) National Centre of Excellence focusing on the regulation of male germ cell differentiation and its impact on infertility and testicular cancer. He is regularly invited to present lectures at national and international meetings. While president of the Australian Society for Medical Research in 2004, he led national advocacy efforts seeking increased federal government funding for health and medical research. He is a member of several international editorial boards. Professor Sinclair has received numerous national and international awards including the Outstanding Research Award from the Royal Society UK and the 2009 Sutherland Award for his contributions to Human Genetics. In 2005 he was appointed to the Australian Biotechnology Advisory Council and has been chair of the NHMRC Grant Review Panel for Genetics since 2007. In 2009, he was appointed by the Federal Minister for Health to advise the government on issues relating to human genetics.

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
2007 Rudolf Jaenisch
2008 Haifan Lin
2009 Blanche Capel
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. Dolores Lamb of Baylor College of Medicine as the 2010 Distinguished Andrologist for her outstanding contributions in the realm of male infertility and her exceptional influence on the development of andrology clinicians and scientists throughout her career.

Dr. Lamb received her BA in biology (magna cum laude) from Molloy College in New York in 1974, and her PhD from the University of Texas in 1980 with her dissertation on androgens in the Sertoli cell. She then went on to join Baylor College of Medicine, where she rose rapidly through the academic ranks, and was named full tenured professor in 2003, with joint appointments in the Departments of Molecular and Cellular Biology and the Department of Urology. In 2006, she was awarded an endowed chair as the "Lester and Sue Smith Chair of Basic Urologic Research " and vice chairman for research in the Department of Urology.

Dr. Lamb is best known for her research in molecular mechanisms of male infertility. This started with her graduate work on the Sertoli cell followed by her independent studies of the regulation of spermatogenesis. She then went on to do cutting edge studies on sperm aneuploidy and the role of DNA repair defects in the sperm from men with severe oligospermia or non-obstructive azoospermia, where she is the current thought leader on the genetic risks to offspring of men with severe underproduction undergoing IVF/ICSI to achieve pregnancy. In parallel, she has studied as other aspects of men’s health with clinical import, particularly prostate biology with a focus on the androgen receptor and inhibitors of prostate cancer cell growth.

Dr. Lamb’s productivity is phenomenal. Her prodigious funding from various sources including NIH, Dept. of Defense and Prostate Cancer Foundation attests to her ongoing activity. She has published over 200 peer-reviewed manuscripts and innumerable reviews, seminars and syllabi. This all while she maintains and supervises a busy clinical fertility laboratory.

Dr. Lamb’s contribution to the field of andrology went beyond research. She has left a clear mark in the clinical field of male infertility, where she has probably trained a solid majority of urologists with an active research program in andrology. Many of her trainees are now heads of their divisions in various urological training programs across the country. In addition to the presidency of the American Society of Andrology, she has been in leadership positions in many societies such as the Society of Male Reproduction and Urology, the American Society of Reproductive Medicine, the American Urological Association (AUA) and the Endocrine Society. She is an excellent lecturer who is extremely sought after, for programs with a clinical or basic science bent alike.

Dr. Lamb’s dedication to the field is attested to by her service to the ASA. In addition to her service as president of the society, she has been chair of the program committee in 1997, 2005 and 2009, secretary from 1998 to 2001, and has been on the executive council for many years.

In recognition of Dr. Lamb’s many accomplishments, the ASA is proud to select her as the Distinguished Andrologist for 2010.

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. Elk-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
2007  Eberhard (Ebo) Nieschlag
2008  Bernard Robaire
2009  William Bremner

The Distinguished Andrologist Award is sponsored by the American Society of Andrology.
The Distinguished Service Award is bestowed annually to recognize an individual who has provided distinguished service to the American Society of Andrology. This year’s recipient is Dr. Joel Marmar, of Cooper University Hospital.

Dr. Marmar, a charter member of the ASA, has provided valuable service to the society for a long time. He served as secretary for six years back in the “old days” when there was no business office. The amount of effort this required cannot be understated, and has been described by veteran council members as “huge”, “arduous” and “herculean”. In 1983, he served as both the program chair and local arrangements chair for the annual meeting in Philadelphia. Again, this was done without the assistance of a business office. Not only did Dr. Marmar reserve hotel space, negotiate rates, arrange for food and entertainment, he also modernized the meeting booklet for that meeting, adding many sections still used today. That meeting was widely considered a big success. He reprised his role as local arrangements chair in 2009.

Over the years, he continued serving the society in various roles. He has been elected to the executive council, and has served as Finance Committee chair, and Journal of Andrology editorial board member.

More recently, he has been recognized throughout the society for his drive and effort in organizing the Andrology Self Assessment Exam. This project was borne out of the effort to allow both clinical and basic science andrologists to obtain certification in andrology, and required a prodigious effort on Dr. Marmar’s part as liaison chair with the American Society of Reproductive Medicine and the American Urological Association. He was also instrumental in obtaining funding for the ASA traveling fellowship program, and acted as liaison with sister societies in co-sponsoring the travel awards.

Dr. Marmar’s quiet manner should not belie his many achievements and his long service to the society. These are all the more remarkable considering he has continued to maintain a full-time medical practice. For these reasons, the ASA is proud to gratefully present him with the 2010 Distinguished Service Award.
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. Peter Liu, MD, PhD of the University of Sydney is this year’s recipient. Dr. Liu obtained his bachelor of medicine and surgery with honors at the University of Sydney in 1993. He became a fellow of the Royal Australasian College of Physicians in 1999. He received his PhD degree at the ANZAC Research Institute University of the University of Sydney in 2003, under the mentorship of David Handelsman.

An accomplished physician-scientist, Dr. Liu has trained at three strong andrology programs and has contributed many papers to the field. His PhD work with Dr. Handelsman on the effects of androgen in older men resulted in 12 papers and 8 chapters. He was equally prolific, if not more, during his two-year post-doctoral fellowship at the Mayo Clinic working with Johannes Veldhuis on neuroendocrine regulation of pituitary and testicular hormones, where he managed to publish over 15 manuscripts and 2 chapters. This pattern continued in 2004 - 2006 when he was working with Ronald Swerdloff on hormonal male contraception. His work on the recovery of spermatogenesis after hormonal contraception led to a landmark paper in Lancet.

Dr. Liu did not slow down upon returning to Australia. He secured two highly competitive grants from the National Health and Medical Research council of Australia, and his current research at the ANZAC and Woolcock institutes is of the highest quality. With his MD and PhD background, his research sits at the interface of basic and clinical research. He has been promoted to associate professor and is actively training PhD students.

Despite being based in Australia, Dr. Liu has been very active at ASA meetings, chairing or co-chairing many sessions. He is on the Membership Committee, where he is promoting growth the international membership of the society. He has been described as an excellent clinician and teacher, and has clearly found that ideal mix of medical practice and basic research that is often sought but rarely achieved. The dearth of MDs among past Young Andrologist recipients testifies to the difficulty involved.

In summary, Dr. Liu has made significant contributions to several subfields of andrology (androgen effects on aging and sleep, Klinefelter syndrome, hormonal contraception among others) in a very short span of time. With such a record, he is certainly destined to be a leader in our field. The ASA is proud to recognize him as this year’s recipient of the Young Andrologist Award.

Young Andrologist Award Recipients

<table>
<thead>
<tr>
<th>Year</th>
<th>Recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>L.J.D. Zaneveld</td>
</tr>
<tr>
<td>1983</td>
<td>William B. Neaves</td>
</tr>
<tr>
<td>1984</td>
<td>Lonnie D. Russell</td>
</tr>
<tr>
<td>1985</td>
<td>Bruce D. Schanbacher</td>
</tr>
<tr>
<td>1986</td>
<td>Stephen J. Winters</td>
</tr>
<tr>
<td>1987</td>
<td>Ilpo T. Huhtaniemi</td>
</tr>
<tr>
<td>1988</td>
<td>Larry Johnson</td>
</tr>
<tr>
<td>1989</td>
<td>Barry T. Hinton</td>
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<tr>
<td>1990</td>
<td>Luis Rodriguez/Rigau</td>
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<tr>
<td>1991</td>
<td>Patricia M. Saling</td>
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</tbody>
</table>

Young Andrologist Award Recipients

<table>
<thead>
<tr>
<th>Year</th>
<th>Recipient</th>
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<tbody>
<tr>
<td>1992</td>
<td>Gary R. Klinefelter</td>
</tr>
<tr>
<td>1993</td>
<td>Robert Chapin</td>
</tr>
<tr>
<td>1994</td>
<td>Wayne J.G. Hellstrom</td>
</tr>
<tr>
<td>1995</td>
<td>Christopher DeJonge</td>
</tr>
<tr>
<td>1996</td>
<td>Paul S. Cooke</td>
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<tr>
<td>1997</td>
<td>Gail A. Cornwall</td>
</tr>
<tr>
<td>1998</td>
<td>William R. Kelce</td>
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<tr>
<td>1999</td>
<td>Stuart E. Ravnik</td>
</tr>
<tr>
<td>2000</td>
<td>Matthew P. Hardy</td>
</tr>
<tr>
<td>2001</td>
<td>Jacquette Trasler</td>
</tr>
</tbody>
</table>

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 2010 Outstanding Trainee Investigator Award will be announced during the Annual Business Meeting on Monday, April 12, 2010 at 5:30 p.m.

Outstanding Trainee Investigator Award Recipients

<table>
<thead>
<tr>
<th>Year</th>
<th>Recipient</th>
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<tbody>
<tr>
<td>1983</td>
<td>Thomas T. Tarter</td>
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<tr>
<td>1984</td>
<td>Peter S. Albertson</td>
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<tr>
<td>1985</td>
<td>Randall S. Zane</td>
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<tr>
<td>1986</td>
<td>Mark A. Hadley</td>
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<tr>
<td>1987</td>
<td>Peter Grosser</td>
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<tr>
<td>1988</td>
<td>Stuart E. Ravnik</td>
</tr>
<tr>
<td>1989</td>
<td>Tracy L. Rankin</td>
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<tr>
<td>1990</td>
<td>Donna O. Bunch</td>
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<tr>
<td>1991</td>
<td>Robert Viger</td>
</tr>
<tr>
<td>1992</td>
<td>John Kirby</td>
</tr>
<tr>
<td>1993</td>
<td>Michael A. Palladino</td>
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</tbody>
</table>

Outstanding Trainee Investigator Award Recipients

<table>
<thead>
<tr>
<th>Year</th>
<th>Recipient</th>
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</thead>
<tbody>
<tr>
<td>1994</td>
<td>Linda R. Johnson</td>
</tr>
<tr>
<td>1995</td>
<td>Mehdi A. Akhondi</td>
</tr>
<tr>
<td>1996</td>
<td>Wei Gu, Daniel B. Rudolph</td>
</tr>
<tr>
<td>1997</td>
<td>Loren D. Walensky</td>
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<tr>
<td>1998</td>
<td>Dolores D. Mruk</td>
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<tr>
<td>1999</td>
<td>Jacques J. Tremblay</td>
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<td>2000</td>
<td>Jeffrey J. Lysiai</td>
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<td>2001</td>
<td>Alexander T.H. Wu</td>
</tr>
<tr>
<td>2002</td>
<td>Etbetsam Attaya</td>
</tr>
<tr>
<td>2003</td>
<td>Mustafa Faruk Usta</td>
</tr>
</tbody>
</table>

2002 | Christopher L.R. Barratt |
2003 | Joanna E. Ellington       |
2004 | Kate Loveland             |
2005 | Janice Bailey             |
2006 | Janice P. Evans           |
2007 | John K. Amory             |
2008 | Moira K. O'Bryan          |
2009 | Michael A. Palladino      |
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)
James Nelson, Ill, MD
Eugenia Rosemberg, MD
Richard Sherins, MD
Anna Steinberger, PhD
West Michigan Reproductive Institute
Texas Institute for Reproductive Medicine and Endocrinology

Silver Level
(Multiple or single contribution(s) greater than or equal to $5,000)
Gail S. Prins, PhD
Susan A. Rothmann, PhD, HCLD
Bayard T. Storey, PhD
J. Lisa Tenover, MD, PhD
Donna L. Vogel, MD, PhD
Christina Wang, MD
Women in Andrology

Sustaining
(Multiple or single contribution(s) greater than or equal to $2,000)
Rupert P. Amann, PhD
Richard D. Amelar, MD
Rudi Ansbacher, MD
Arnold M. Belker, MD
William J. Brenner, MD, PhD
Glenn R. Cunningham, MD
E. Mitch Eddy, PhD
Erwin Goldberg, PhD
Marc Goldstein, MD
Wayne J.G. Hellstrom, MD
Ronald W. Lewis, MD
Bernard Robaire, PhD
Richard J. Sherins, MD
Cheves & Polly Smythe
Terry T. Turner, PhD
Richard Van Clark, MD, PhD

Annual Contributions for Fiscal 2009

$100-$249
John K. Amory, MD
Steven P. Anderson
Martine Culty, PhD
Alan Diekman, PhD
E. Mitch Eddy, PhD
Joh & Berthe Ford
Frank S. French, MD
T.N. Gardner
Wylie C. Hembree, MD
Rex A. Hess, PhD
Barry T. Hinton, PhD
Mary M. Lee, MD
Michael & Anita Lev-Gur
Shelly & Barbara Liss
Marvin L. Meistrich, MD
Robert D. Oates, MD
Sally Perreault Damrey, PhD
Jon Lee Pryor, MD
Allen & Janet Root
Mark Sigman, MD
Donald J. Tindall, PhD
Sheldon & Linda Rubenfeld
Victor & Michelle Sierpina

$250-$999
Rupert P. Amann, PhD
Andrzej Bartke, PhD
Glenn R. Cunningham, MD
Larry I. Lipshultz, MD
Richard J. Sherins, MD
Bayard T. Storey, PhD
J. Lisa Tenover, MD, PhD

$50-$99
Christopher J. De Jonge, PhD, HCLD
Erma Z. Drobnis, PhD
George L. Gerton, PhD
Carin V. Hopps, MD
Sarah Kimmins, PhD
Patricia L. Morris, PhD
Darius A. Paduch, MD, PhD
Dirk F. Propping, MD, PhD
Chantal M. Sottas, BA
Ronald Swerdloff, MD
Manley and Audrey Mandel

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.
Andrology Lab Workshop 2010
“Sperm Morphology: A Hands-On Workshop”

The Andrology Laboratories Committee will present a unique, laboratory-based training workshop with "hands-on" exploration of virtual smears for sperm morphology assessments at the 2010 American Society of Andrology Meeting. 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. The workshop will also include a consensus study on the second day, to use the current classification methods in order to develop agreement on a standard.

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 system and the Strict Criteria (Tygerberg, WHO 4th) 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. Participants will grade images in real time using an audience response system. After the presentation, each participant will receive virtual smears with photographed sperm images to perform a morphology assessment. Participants may keep the virtual smears for future reference and practice.

In the afternoon, results of the analysis of virtual smears will be used to discuss quality control requirements for the laboratory, and to demonstrate how to create and use a QC control chart. You will learn how to improve the quality in your laboratory by reducing variation and by learning to identify and remove special and common causes of becoming overly critical.

The next morning will begin with some background on a previous consensus trial, and an introduction to the methodology and equipment for the consensus study. Each participant will receive packets of virtual smears to classify using multi-voting equipment. Images will be classified in groups, with breaks to analyze data and discuss any controversial images. The workshop will conclude with a summary and moderated discussion. Ample time for discussion of all topics has been allocated.

Learning Objectives
At the conclusion of the Andrology Lab Workshop, participants should be able to:

- Identify the history of sperm morphology and its clinical significance
- Describe and perform sperm classification assessments using WHO 3rd and Strict (Tygerberg, WHO 4th) Criteria
- Distinguish the differences between the two morphology classification systems
- Use and calculate control charts
- Create measures to identify common and special causes of variation and how to reduce them
- Construct a consensus for a morphology standard

Basic Science Workshop at ASA 2010

Objectives
Upon conclusion of the Basic Science Workshop, participants should be able to:

- Identify and compare key techniques, reagents and experimental approaches
- Identify common needs for expansion of technical expertise and reagents to promote best practice and cutting edge science in our field
- Create enhanced communication and interaction between ASA delegates, including trainees

ASA Special Symposium 2010
“Challenges in Urological Andrology”

Needs Assessment
Andrology is a complex scientific, medical, and surgical discipline that encompasses male sexual health, endocrinology, and reproduction. Each of these facets of andrology may present unique and interesting challenges to those dedicated to the field. In this symposium, the lecturers will identify and explore some of the more intricate aspects of andrology. The overriding aim of this program is to provide attendees with a state-of-the-art overview of andrological “challenges” and solutions as presented by the expert participating faculty.

Learning Objectives
At the conclusion of the Special Symposium, participants should be able to:

- Describe novel concepts in treating hypogonadism and optimizing sperm production
- Identify the clinical impact of new insights into the processes of sperm transport and capacitation
- Review contemporary treatment modalities for erectile dysfunction and premature ejaculation
- Explain approaches to prevent penile shortening and optimize patient satisfaction after penile prosthesis implantation
- Illustrate tissue engineering techniques used in the treatment of small penis syndrome

35th Annual ASA Meeting
“Lifetime Determinants of Male Reproductive Health”

Needs Assessment
Normal male reproductive function is very sensitive to a variety of potential insults. Current evidence suggests that overall male reproductive health may be declining. Normal reproductive development requires a proper embryologic, childhood, and adult environment. A variety of disturbances throughout the life cycle may result in dysfunction of the reproductive system. Evidence from both animals and humans has lead to insights into normal and abnormal reproductive function. In addition, links between reproductive dysfunction and systemic illnesses such as cardiovascular disease have been documented. Current practice guidelines require detailed reproductive, developmental, and lifestyle histories be obtained during the evaluation of reproductive dysfunction. This requires knowledge of normal reproductive function and development as well as potential causes of abnormal function. To obtain this knowledgebase, interaction between clinicians and basic scientists is mandatory. This will allow clinicians to learn the latest data on function and dysfunction. In addition, basic scientists may become aware of the areas in which more research is needed.
The program will present information for clinicians and scientists on normal and abnormal reproductive function from embryologic development through advanced age. The interaction between clinicians and scientists encourages advances in scientific understanding. In addition, the increased understanding facilitates improved patient care and hopefully to advances in treatment.

**Learning Objectives**
- Describe the use of whole genome sequencing to explore disorders of testis development
- Explain the relationship between epigenetic reprogramming of germ cells and testis cancer
- Explain the relationship between fetal-neonatal development and future fertility in males
- Identify the relationship between ED and cardiometabolic dysfunction
- List some of the genes involved in coordination of somatic and germ cell development
- Describe how the erection and ejaculation occurs, and identify the key defects that contribute to ejaculatory defects
- Describe normal and abnormal pubertal patterns
- Recognize the effects of obesity and cannaboids on fertility in the male
- List the potential effects of aging and environmental insults on the sperm genome

**ASA 36th Annual Meeting**
April 2 – 5, 2011
Hyatt Regency Montreal
Montreal, Canada

**XXI Testis Workshop**
March 30 – April 2, 2011

**Andrology Lab Workshop**
April 2 – 3, 2011
# Schedule of Events

## Andrology Lab Workshop
### Sperm Morphology: A Hands-On Workshop
**Saturday, April 10 – Sunday, April 11, 2010**

**Chair:** Dean Morbeck, PhD, HCLD  
**Location:** Regency A

### FRIDAY, APRIL 9, 2010

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:00 p.m. – 6:00 p.m.</td>
<td>Registration/Information Desk Open</td>
<td>Regency Foyer</td>
</tr>
<tr>
<td>7:00 a.m. – 7:00 p.m.</td>
<td>Registration/Information Desk Open</td>
<td>Regency Foyer</td>
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<tr>
<td>4:00 p.m. – 9:30 p.m.</td>
<td>Exhibit Hall Open</td>
<td>Colonnade Salon</td>
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<tr>
<td>8:00 a.m. – 8:30 a.m.</td>
<td>Continental Breakfast</td>
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<tr>
<td>8:30 a.m. – 9:15 a.m.</td>
<td>Overview of Sperm Morphology Classification Systems</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>9:15 a.m. – 10:00 a.m.</td>
<td>Interactive Instruction – WHO 3rd Edition and Strict/WHO 4th Edition Classification Methods</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>10:00 a.m. – 11:30 a.m.</td>
<td>Exercise 1 – Sperm Morphology</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>11:45 a.m. – 1:15 p.m.</td>
<td>Laboratory Science Forum Luncheon (included)</td>
<td>Karolinska University Hospital</td>
</tr>
<tr>
<td>1:30 p.m. – 2:15 p.m.</td>
<td>Exercise 2 – Sperm Morphology</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>2:15 p.m. – 3:00 p.m.</td>
<td>Quality Control – Understanding QC Requirements and Construction &amp; Use of Control Charts</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>3:00 p.m. – 3:15 p.m.</td>
<td>Break</td>
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### SATURDAY, APRIL 10, 2010

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<td>Regency Foyer</td>
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<td>7:00 a.m. – 6:00 p.m.</td>
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### SUNDAY, APRIL 11, 2010

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<th>Location</th>
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<tbody>
<tr>
<td>7:00 a.m. – 7:00 p.m.</td>
<td>Continental Breakfast</td>
<td>Colonnade Salon</td>
</tr>
<tr>
<td>8:00 a.m. – 8:10 a.m.</td>
<td>Introduction</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>8:10 a.m. – 9:00 a.m.</td>
<td>Morphology Consensus Study Part I</td>
<td></td>
</tr>
<tr>
<td>9:00 a.m. – 9:30 a.m.</td>
<td>Distinguished Service Award/Break</td>
<td>Regency Ballroom DEFG</td>
</tr>
<tr>
<td>9:30 a.m. – 10:00 a.m.</td>
<td>Discussion of Data &amp; Controversial Images</td>
<td>Fertility Solutions Inc.</td>
</tr>
<tr>
<td>10:00 a.m. – 10:30 a.m.</td>
<td>Morphology Consensus Study Part II</td>
<td></td>
</tr>
<tr>
<td>10:30 a.m. – 10:45 a.m.</td>
<td>Break – Visit Exhibits</td>
<td>Colonnade Salon</td>
</tr>
<tr>
<td>10:45 a.m. – 11:45 a.m.</td>
<td>Discussion of Consensus Data</td>
<td>Fertility Solutions Inc.</td>
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<tr>
<td>11:45 a.m. – 12:00 p.m.</td>
<td>Open Discussion of Future Work, Recommendations</td>
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</tbody>
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## Basic Science Workshop
**Saturday, April 10, 2010**

**Chair:** Kate Loveland, PhD  
**Location:** Regency C

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tbody>
<tr>
<td>2:00 p.m. – 6:00 p.m.</td>
<td>Registration/Information Desk Open</td>
<td>Regency Foyer</td>
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<tr>
<td>7:00 a.m. – 7:00 p.m.</td>
<td>Registration/Information Desk Open</td>
<td>Regency Foyer</td>
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<tr>
<td>4:00 p.m. – 9:00 p.m.</td>
<td>Exhibit Hall Open</td>
<td>Colonnade Salon</td>
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**ASA Special Symposium**

**Challenges in Urological Andrology**

*Saturday, April 10, 2010*

Program Chairs: Robert Brannigan, MD and Run Wang, MD

Location: Regency Ballroom DEFG

Registration is complimentary; however, limited seating is available.

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**SATURDAY, APRIL 10, 2010**

1:00 p.m. – 1:20 p.m.  **Premature Ejaculation: Overview of Available Therapies**  
Wayne J.G. Hellstrom, MD  
Tulane University School of Medicine

1:20 p.m. – 1:40 p.m.  **Therapies for Erectile Dysfunction and their Effect on Endothelium**  
John Mulhall, MD  
Memorial Sloan-Kettering Cancer Center

1:40 p.m. – 2:00 p.m.  **Q & A**

2:00 p.m. – 2:20 p.m.  **Peyronie’s Disease: What are the Risk Factors?**  
Mohit Khera, MD  
Baylor College of Medicine

2:20 p.m. – 2:40 p.m.  **Novel Concepts in Managing Hypogonadism and Improving Spermatogenesis**  
Larry I. Lipschultz, MD  
Baylor College of Medicine

2:40 p.m. – 3:00 p.m.  **Q & A**

3:00 p.m. – 3:20 p.m.  **The Long Journey: Sperm Transport and Capitation**  
Erma Z. Drobnis, PhD  
Missouri Center for Reproductive Medicine & Fertility

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**FRIDAY, APRIL 9, 2010**

2:00 p.m. – 6:00 p.m.  **Registration**  
Location: Regency Ballroom Foyer

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**SATURDAY, APRIL 10, 2010**

✓ 7:00 a.m. – 7:00 p.m.  **Welcome and Opening Remarks**

6:00 p.m. – 6:10 p.m.  **Welcome and Opening Remarks**

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

✓ 6:30 p.m. – 7:30 p.m.  **EMIL STEINBERGER MEMORIAL LECTURE**  
**Insights into Disorders of Testis Development Using Whole Genome Analysis**  
Andrew Sinclair, PhD  
Murdoch Children’s Research Institute, Australia  
(Introduced by Dolores Lamb, PhD)

7:30 p.m. – 7:50 p.m.  **Updates from NICHD & NIEHS**  
Stuart B. Moss, PhD  
NICHD

Jerry J. Heindel, PhD  
NIEHS

7:50 p.m. – 9:30 p.m.  **Welcome Reception**  
Location: Colonnade Salon

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ASA 35th Annual Meeting  
Lifetime Determinants of Male Reproductive Health  
April 10 – 13, 2010  
Program Chairs: Kate Loveland, PhD and Mark Sigman, MD

Location: Regency Ballroom DEFG

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

*Please note that the all sessions will be held in Regency Ballroom DEFG unless otherwise noted.
SUNDAY, APRIL 11, 2010

6:30 a.m. - 8:00 a.m. Past President's Breakfast
Location: Woodway

7:00 a.m. - 4:00 p.m. Exhibit Hall Open
Location: Colonnade Salon

7:00 a.m. - 6:00 p.m. Registration
Location: Regency Ballroom Foyer

8:00 a.m. - 9:00 a.m. AUA LECTURE
Melding Basic Research With Clinical Inference Needs — A Glass Half Empty or Half Full
Larry I. Lipshultz, MD
Baylor College of Medicine
(Introduced by Mark Sigman, MD)

8:00 a.m. - 9:00 a.m. Poster Session I
Location: Colonnade Salon

9:00 a.m. - 9:15 a.m. Distinguished Service Award

9:15 a.m. - 10:45 a.m. SYMPOSIUM I - Life in the Womb: Fetal Determinants of Men's Reproductive Health
Co-Chairs: John H. Richburg, PhD
Robert D. Oates, MD
Epigenetic Reprogramming of Germ Cells and Relevance to Testicular Cancer
Sarah Kimmis, PhD
McGill University
Animal Models for Testicular Cancer
D.N. Rao Veeramachaneni, DVM, PhD
Colorado State University
Relevance of Human Testis Fetoepiagenet
Development for Future Fertility
Hector E. Chemes, MD, PhD
Buenos Aires Children's Hospital, Argentina

10:45 a.m. - 11:00 a.m. Break — Visit Exhibits
Location: Colonnade Salon

11:00 a.m. - 12:30 p.m. Poster Session I
Location: Colonnade Salon

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: Windsor
“From Mentee to Mentoring: Preparing Women for Success in Science Careers”
Gayle Slaughter, PhD
Baylor College of Medicine

1:00 p.m. - 3:30 p.m. Oral Session I — Basic Science
Location: Regency Ballroom B
Moderators: Ina Dobrinski, DVM, PhD
Greg Buchold, PhD

2:00 p.m. - 3:30 p.m. Oral Session II — Clinical
Location: Regency Ballroom C
Moderators: John K. Amory, MD
Shayne Louis, MD

2:30 p.m. - Abstract 3
ELMO1 FUNCTIONS IN SERTOLI CELL-MEDIATED APOPTOTIC GERM CELL PHAGOCYTOSIS AND HOMEOSTASIS IN THE TESTES
Michael Elliott, PhD, Daeho Park, PhD, Robin Woodson, Shuqiu Zheng, PhD, Michael Reardon, Kodi Ravichandran, PhD and Jeffrey Lysiak, PhD
(Presented By: Michael Elliott, PhD)

2:45 p.m. - Abstract 4
TESTICULAR LUMINAL FLUID FACTORS REGULATE MAPK, JAK/STAT AND NFkB PATHWAYS IN THE INITIAL SEGMENT OF RAT EPIDIDYMIS TO PREVENT CELL APOPTOSIS
Bingfang Xu, Rana Abdel-Fattah, Ling Yang, Sallie Crenshaw and Barry Hinton
(Presented By: Bingfang Xu)

3:00 p.m. - Abstract 5
FERTILIZATION DEFECTS IN SPERM FROM TYROSYLPROTEIN SULFOTRANSFERASE 2 (TPST2)-DEFICIENT MICE ARE LINKED WITH ABNORMALITIES IN A DISINTTEGRIN AND A METALLOPROTEASE (ADAM) PROTEINS AND NOT ABNORMALITIES IN IZUMO
Matthew Marcello, Welpa Jia, PhD, Julie Leary, PhD, Kevin Moore, MD and Janice Evans, PhD
(Presented By: Matthew Marcello)

3:15 p.m. - Abstract 6
ROLE OF ENKURIN IN MALE FERTILITY
Melissa Jungnickel, PhD, Keith Sutton, PhD and Harvey Fierman, PhD
(Presented By: Melissa Jungnickel, PhD)

3:45 p.m. - Abstract 7
ANDROGEN-SPECIFIC REGULATION OF CYP24 IN HUMAN PROSTATE CANCER CELL LINES
Sarmistha Mukherjee, PhD, Aysegul Sahin, BS (Biology), Josephine Addai, BS (Microbiology), Julie N. Stewart MD and Dolores J. Lamb, PhD
(Presented By: Sarmistha Mukherjee, PhD)

3:45 p.m. - Abstract 8
RELIABILITY OF ANOGENITAL DISTANCE MEASUREMENT AS AN ASSAY OF MALE FERTILITY
Akanksha Mehta, MD, Kim Boekelheide, PhD and Mark Sigman, MD
(Presented By: Akanksha Mehta, MD)

4:00 p.m. - Abstract 9
GENETIC AND HORMONAL CONTROL OF BONE VOLUME, ARCHITECTURE AND HISTOMORPHOMETRY IN XXV MICE
Peter Liu, MBBS, (Hons) PhD, Robert Kaliak, PhD, Yan-Hee Lue, PhD, Krista Erkki, MD, PhD, Yue Jia MD, Hong Zhou, PhD, Markus Seibel, MD, Christina Wang, MD, Ronald Swerdloff, MD and Colin Dunstan, PhD
(Presented By: Peter Liu, MBBS, (Hons) PhD)

4:45 p.m. - Abstract 10
ENHANCEMENT OF VITAMIN D BASED THERAPY FOR PROSTATE CANCER TREATMENT BY SELECTIVE TARGETING OF 24-HYDROXYLASE
Mounia Tannour-Louet, PhD, Shaye Lewis, PhD, Julie Stewart, MD, Aysegul Sahin, BS, Shuo Han, BS, Josephine Addai, BS and Dolores Lamb, PhD
(Presented By: Mounia Tannour-Louet, PhD)

4:45 p.m. - Abstract 11
RHOA/RHOKINASE-MEDIATED CA2+ SENSITIZATION AND CA2+ ENTRY VIA L-TYPE VOLTAGE-GATED CA2+ CHANNELS MAINTAIN HUMAN CAVERNOSAL RESISTANCE BY ACTING DIRECTLY ON NORADRENERGIC NEURONS IN THE FLACCID STATE
Serpur Gur, PhD, Philip Kadowitz, PhD, Suresh Sikka, PhD and Wayne Helstrom, MD, FACS
(Presented By: Suresh Sikka, PhD)

*Locations subjects to change
3:15 p.m. – Abstract 12
IDENTIFICATION OF COPY NUMBER VARIANTS ASSOCIATED TO GENITOURINARY BIRTH DEFECTS AND CHARACTERIZATION OF NOVEL KEY GENES IN HUMAN GENITAL DEVELOPMENT
Shuo Han, Mounia Tannour-Louet, PhD, Sean Corbett, MD and Dolores Lamb, PhD (Presented By: Shuo Han)

3:30 p.m. – 4:00 p.m.  Break
Location: Colonnade Salon

4:00 p.m. – 4:45 p.m.  LECTURE I
Erectile Dysfunction: A Red Flag for Early Cardiometabolic Dysfunction
Martin Miner, MD
Brown University
(Introduced by J. Lisa Tenover, MD, PhD)

4:45 p.m. – 5:30 p.m.  LECTURE II
The Progression to Androgen-Independent Prostate Cancer
Robert J. Matusik, PhD
Vanderbilt University
(Introduced by Gail S. Prins, PhD)

6:30 p.m. – 8:30 p.m.  Trainee Forum and Mixer
(All Trainee Travel Awards will be distributed and celebrated at this event)
Location: Regency Ballroom ABC

Monday, April 12, 2010

7:00 a.m. – 4:00 p.m.  Exhibit Hall Open
Location: Colonnade Salon

7:00 a.m. – 6:00 p.m.  Registration
Location: Regency Ballroom Foyer

8:00 a.m. – 9:00 a.m.  WOMEN IN ANDROLOGY LECTURE
Epigenetic Changes in Sperm: A Cause of Male Factor Infertility
Rebecca Z. Sokol, MD, MPH
University of Southern California
(Introduced by Moira K. O’Brien, BSc, PhD)

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

9:15 a.m. – 10:45 a.m.  SYMPOSIUM II – Size Does Matter!
Growth in the Juvenile
Co-Chairs: Martin M. Matzuk, MD, PhD
Catherine Ittmann, PhD

Doublesex-Related Genes Coordinate Somatic and Germ Cell Development in the Mammalian Testis
David Zarkower, PhD
University of Minnesota

Vitamin A and the Onset of Meiosis
Michael D. Griswold, PhD
Washington State University

Normal and Abnormal Pubertal Patterns and Their Regulation
Mary M. Lee, MD
University of Massachusetts

10:45 a.m. – 11:00 a.m.  Break – Visit Exhibits
Location: Colonnade Salon

11:00 a.m. – 12:30 p.m.  Poster Session II
Location: Colonnade Salon

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 AND TRAINEE AFFAIRS COMMITTEES
(Not included in registration; tickets required)
Location: Regency Ballroom C
“So You Want to Write a Grant Application to the NIH: How the Program Officer and Scientific Review Officer Can Help”
Stuart B. Moss, PhD
NICHD

12:30 p.m. – 2:00 p.m.  Editorial Board Luncheon
Location: Regency Ballroom AB

2:00 p.m. – 3:30 p.m.  SYMPOSIUM III – Fertility in Adult Life
Co-Chairs: Jeffrey J. Lysiak, PhD
Cigdem Tanrikut, MD

Roles of Testicular MicroRNAs
Wei Yan, MD, PhD
University of Nevada

Sperm and Your Natural Marijuana: Endocannabinoids, Nicotine and Fertility
Lani Burkman, PhD
University of Buffalo

Phosphorylation Events During Sperm Capacitation
Pablo E. Visconti, PhD
University of Massachusetts

Male Reproductive Tract Function
Bernard Robaire, PhD
McGill University
(Introduced by Joseph Alukal, MD)

4:00 p.m. – 4:45 p.m.  LECTURE IV:
Translational Andrology: Bridging Bench and Bedside
Christina Wang, MD
UCLA Medical Center
(Introduced by William J. Bremner, MD, PhD)

5:30 p.m.  ASA Business Meeting

7:00 p.m. – 11:00 p.m.  Annual Banquet
(Not included in registration fee; tickets required)
Location: Black Swan Nightclub (on property)

Outstanding Trainee Investigator and Trainee Awards
TUESDAY, APRIL 13, 2010

7:00 a.m. – 8:00 a.m.  2010 Program Committee Meeting
  Location: Westbury

7:30 a.m. – 12:15 p.m.  Registration
  Location: Regency Ballroom Foyer

8:00 a.m. – 9:30 a.m.  SYMPOSIUM IV – Aging and Male Reproductive Health
  Co-Chairs:  Kirk C. Lo, MD, FRCSC
  Douglas Stocco, PhD

  Effects of Aging and Environmental Insults on the Sperm Genome
  Andrew J. Wyrobek, PhD
  Lawrence Berkeley Labs

  Testosterone and the Aging Male
  Alvin M. Matsumoto, MD
  University of Washington

  The Relationship of Paternal Age to Health and Disease in Offspring
  Susan Harlap, MD
  New York University

9:30 a.m. – 9:45 a.m.  Break – Visit Exhibits
  Location: Colonnade Salon

9:45 a.m. – 12:15 p.m.  INTERNATIONAL LECTURE:
  Mechanisms of Fertilization – A View Through Gene Manipulated Mice
  Masaru Okabe, PhD
  Osaka University
  (Introduced by Patricia S. Cuasnicu, PhD)

10:45 a.m. – 12:15 p.m.  SYMPOSIUM V – Long-Lasting Sex
  Co-Chairs:  Marvin L. Meistrich, PhD
  Ajay K. Nangia, MBBS

  The Therapeutic Potential of Germ Stem Cells
  Amander Clark, PhD
  University of California, Los Angeles

  Love and Monogamy: An Endocrine Story
  C. Sue Carter, PhD
  University of Illinois at Chicago

  Long-Lasting S-E-X (Science, Education, Xenogamy)
  Anna Steinberger, PhD
  Professor Emerita, UTMS--Houston

MEETING ADJOURNED
EMIL STEINBERGER MEMORIAL LECTURE
Insights into Disorders of Testis Development Using Whole Genome Analysis
Andrew Sinclair, PhD
Murdoch Children's Research Institute and Dept. of Pediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Australia
(Presented By: Andrew Sinclair, PhD)

Disorders of sex development (DSD), ranging in severity from genital abnormalities to complete sex reversal, represent a major clinical concern. The cause of these disorders is most often a disruption of the genetic programs that regulate development of testes or ovaries. Although a number of genes have been identified in these developmental pathways, in many cases of DSD the causative mutations cannot be identified. We have used Affymetrix Genome-Wide Human SNP Array 6.0 to perform whole genome copy number analysis on genomic DNA from 30 individuals with unexplained DSD. Copy number changes identified rearrangements that affected known and novel gonad genes. Known genes affected included: a duplication of the DAX1 gene on the X chromosome of a 46,XY DSD gonadal dysgenesis patient and a deletion in the upstream regulatory region of the SOX9 gene in a 46,XY DSD gonadal dysgenesis individual. Amongst other potentially causative findings were rearrangements affecting the X-linked SOX3 gene in two unrelated 46,XX testicular DSD patients. This is the first evidence in humans that SOX3 may be functionally interchangeable with the mammalian sex-determining gene SRY. These analyses give new insights into the molecular genetic pathways underlying human gonad development and dysfunction. Our data have generated a number of new candidate genes for future mutation screening and suggest that a significant proportion of gonadal dysgenesis cases may be explained by rearrangements that affect the regulatory regions of known gonad determining genes. Funded by an Australian Government National Health and Medical Research Council grant. 

SUNDAY, APRIL 11, 2010
9:15 a.m. - 10:45 a.m.
SYMPOSIUM I – Life in the Womb: Fetal Determinants of Men's Reproductive Health: Epigenetic Reprogramming of Germ Cells and Relevance to Testicular Cancer
Sarah Kimmins and Romain Lambrot
Departments of Animal Science and Pharmacology and Therapeutics, McGill University, Montreal Canada
(Presented By: Sarah Kimmins)

Background and Objectives: The epigenetic layer is comprised of biochemical modifications on histones and methylation of DNA. In spermatogenesis the epigenetic layer is highly dynamic and regulates gene expression during this complex cell differentiation process. Recent studies highlight the role of the epigenome in development and disease, and indicate that gametogenesis may be particularly susceptible to the introduction of epigenetic errors. Strikingly, aberrant epigenetic profiles in the form of anomalous DNA and histone modifications are characteristic of cancerous cells. To date, little information is available on the role of histone modifications in aberrant gene expression in testicular cancer. Our objectives were to investigate the mechanisms by which histone methylation regulates gene expression in testis cancer.

Methods: We defined the epigenetic status of key genes implicated in the pathogenesis of non-seminoma namely the proto-oncogene OCT4 which is over-expressed in testis cancer, and the tumor suppressor RASSF1A which is aberrantly silenced. Cell lines representative of non-seminoma were treated with a chromatin modifying drug to reveal the underlying epigenetic signatures at the OCT4 and RASSF1A promoters. The patterns of histone methylation following treatment were examined using chromatin immunoprecipitation and real time PCR.

Results: Treatment with 5-aza-dC restored RASSF1A expression through a loss of gene silencing histone H3 methylation at lysine 9 (K9), and by retention of gene activating histone H3 lysine 4 (K4) tri-methylation in the promoter region. In contrast the expression of OCT4 was reduced by 5-aza-dC and was associated with a loss of gene activating histone H3-K4 di-methylation in the promoter region. Analysis of DNA methylation revealed a slight reduction in DNA hypermethylation at the RASSF1A promoter, whereas the OCT4 promoter was mostly unmethylated and unaffected.

Conclusions: Aberrant histone modifications may serve as a principle means of deregulation of RASSF1A and OCT4 expression in testis cancer. Understanding the protein players and the meaning of epigenetic modifications in testis cancer will have translational outcomes towards understanding the etiology and prevention of this disease.
Although testicular tumors occur in all domesticated animals, a suitable laboratory animal model is lacking for human testicular cancer. Recent advances in human germ cell cancers include molecular characterization of the likely precursors—transformed gonocytes; these lesions are called carcinoma in situ (CIS), intratubular germ cell neoplasia or transepithelial intratubular neoplasia. We documented CIS-like lesions in horses, deer, and rabbits in association with seminomatous lesions suggesting a progression of events similar to that in humans. Our recent studies in rabbits show that developmental exposure to common pollutants such as DDT induces lesions similar to CIS in men. Cryptorchidism is considered as a significant risk factor for testicular cancer. As CIS was reported to be existent in only 2–4% of cryptorchid men, it is likely that etiological factors that impair testis descent do not invariably transform germ cells. Indeed, we have shown in rabbits that abdominal location per se does not cause germ cell atypia indicating that cellular transformations depend upon the etiology of cryptorchidism. These experimental paradigms will be discussed along with a study involving a unique wild deer population in Alaska, likely exposed to environmental endocrine-disrupting agents. 75% of these deer are cryptorchid and manifest tumors involving germ cells as well as Leydig and Sertoli cells. Testicular tumors involving all three cell types also occur in dogs; they occur with about equal frequency in aged dogs. Observations from an archive related to testicular disorders will be discussed: 1) Hypogonadotrophic hypogonadism or endocrine disruptors may compromise SC proliferation. Testicular mitotic activity in the neonatal rat is critically dependent on FSH. This requirement is also likely to exist in humans, hence recent trials of neonatal treatment with FSH when Hypogonadotrophic Hypogonadism is suspected. 2) In testicular dysgenesis there is failed GC maturation and persistence of embryonic gonocytes with possible transformation to intratubular GC neoplasia that may evolve to GC cancer in adulthood. 3) The diminution of GC numbers during infancy in cryptorchidism is probably primary or dysgenetic in nature and not, as previously believed, a consequence of GC degeneration from early infancy due to the non-scrotal testicular position.
SUNDAY, APRIL 11, 2010
4:45 p.m. – 5:30 p.m.

LECTURE II
The Progression to Androgen-Independent Prostate Cancer
Robert J. Matusik, PhD
Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN
(Presented By: Robert J. Matusik, PhD)

Objectives: Treatment for prostate cancer (PCa) relies upon blocking androgen receptor activity. Eventually, all patients will fail this therapy and the cancer progresses to Androgen-Independence (AI) or Castrate Resistant Prostate Cancer. A central theme now emerging is that AI PCa is still dependent upon androgen receptor (AR) signaling but the pathway(s) that maintains AR addiction is unclear.

Methods: We have shown that the secretion of neuropeptides by neuroendocrine (NE) cells contribute to PCa progression to AI by increasing AR levels and activity. These NE secretions activated Nuclear Factor kappa B (NF-kappaB) in LNCaP cells permitting xenograft growth in castrated mice. To study the NF-kappaB pathway in mice, we used a haploid insufficient IAPPalpha (the inhibitor of NF-kappas) mouse model. Also, since nuclear beta-Catenin can enhance AR activity, we tested if constitutive activation of Wnt/beta-Catenin signaling would prevent the mouse prostate from regressing after castration. Constitutive expression of nuclear beta-catenin was accomplished in transgenic mice with a prostate specific epithelial cell targeted Cre that excised a floxed exon3 from beta-catenin. Removal of exon3 prevents phosphorylation of beta-catenin resulting in the accumulation of nuclear beta-catenin and constitutive activation of the Wnt-signaling pathway.

Results: The activation of NF-kappaB in the mouse prostate results in a stromal and epithelial hyperplasia with histology similar to that seen in human Benign Prostatic Hyperplasia. Further, in castrated mice, the prostate maintains high levels of nuclear AR and shows continued cell proliferation of the prostate gland. In order to test the role of NF-kappaB during progression of cancer, we used the ARR PB-Hi-myC (Hi-myC) transgenic model of prostatic adenocarcinoma. In the Hi-myC mouse, the adenocarcinoma regress after castration, nuclear AR levels remained low, and the tumor does not regrow. However, castrated mice that expressed both NF-kappaB and myc in the prostate did not regress and nuclear AR levels increased. These experiments demonstrated that in vivo expression of NF-kappaB was sufficient to maintain prostate cancer proliferation at castrate levels of androgens. Also, to test the role of Wnt/beta-Catenin signaling during prostate regression, we constitutively activated the expression of nuclear beta-catenin in the prostatic epithelium. This resulted in the appearance of high grade prostate intraepithelial neoplasia that fails to regress after castration.

Conclusions: These results indicate that individually the activation of NF-kappaB or Wnt signaling pathways can maintain AI growth of prostate cancer. In patients where both pathways would be activated, we would expect a rapid failure to androgen ablations therapy. Thus, in patients that have failed androgen blockade, these pathways may be a potential targets for therapy.

Funding was provided by grants from NIDDK (R01-DK0555748), NCI (R01-CA76142), and DoD (W81XWH-08-1-0547).

MONDAY, APRIL 12, 2010
8:00 a.m. – 9:00 a.m.

WOMAN IN ANDROLOGY LECTURE
Epigenetic Changes in Sperm: A Cause of Male Factor Infertility
Rebecca Z. Sokol, MD, MPH
Professor of Medicine and Obstetrics and Gynecology, Keck School of Medicine, University of Southern California
(Presented By: Rebecca Z. Sokol, MD, MPH)

The relationship between epigenetic changes in spermatozoa and male factor infertility is a promising new area of research in the area of male reproduction. Epigenetic programming (altering heritable biological information without changing DNA nucleotide sequence) is a normal process that leads to modifications of gene activity that can be transmitted to daughter cells. One epigenetic process, DNA methylation, appears to influence numerous cellular processes. DNA methylation occurs when a methyl group is covalently bound to the cytosine residue in a CpG sequence of DNA, a reaction catalyzed by DNA methyltransferases. Both de novo methylation of DNA and removal of methyl groups ("erasure") change the epigenetic state. Hypermethylation of CpG islands usually leads to a loss of gene expression. The inheritance or persistence of these epigenetic modifications is referred to as epigenetic reprogramming. Extensive epigenetic reprogramming occurs during normal maturation of germ cells and spermatogenesis.

Improper epigenetic reprogramming can have adverse health effects. Preliminary animal studies suggest that abnormalities of DNA methylation can impact male fertility. Male murine germ cells have highly unique patterns of DNA methylation, which are absent in an infertile male mouse model. Toxic exposures that negatively impact semen parameters also alter sperm DNA methylation in mice. Recent clinical studies indicate that levels of DNA methylation are abnormal in spermatozoa of men who have abnormal sperm concentration, motility, and morphology. Data relating epigenetic changes in spermatozoa to fertility outcomes in the IVF setting, while suggestive, requires more research.
SYMPOSIUM II - Size Does Matter! Growth in the Juvenile
Vitamin A and the Onset of Meiosis
Michael D. Griswold, PhD, Elizabeth Snyder, Cathryn Hogarth, PhD and Chris Small
School of Molecular Biosciences, Washington State University, Pullman, WA
(Presented By: Michael D. Griswold, PhD)

It has been known for many years that Vitamin A in the form of retinoic acid is required for normal spermatogenesis. The objective of this study was to describe the role of retinoic acid in detail and to determine the role of the testicular cell types in its metabolism and activity. The primary activity of retinoic acid in spermatogenesis is in the regulation of the transition of undifferentiated spermatogonia (A spermatogonia) into the differentiation pathway (A1 spermatogonia). The genes Stra8 and Rec8 and other genes that are involved in meiosis are regulated by retinoic acid and allow the entry of germ cells into meiosis. Access to retinoic acid is carefully controlled and limiting in the testis in vivo. This controlled access allows for the generation of the spermatogenic wave and continuous sperm production. The first detectable action of retinoic acid on germ cells can be seen in the mouse shortly after birth. This action is confined to specific regions of the tubule and to specific spermatogonia. This first detectable activity appears to be the harbinger of the asynchronous spermatogenic wave. Thus, vitamin A in the form of retinoic acid is essential for the onset of asynchronous spermatogenesis and for the entry of germ cells into meiosis. Retinoic acid should be considered along with testosterone and FSH as a major regulatory factor of spermatogenesis.

Supported by grants R37 HD10808-31 and U54 HD 42454-06 from NIH

SYMPOSIUM III - Size Does Matter! Growth in the Juvenile
Normal and Abnormal Pubertal Patterns and their Regulation
Mary M. Lee, MD
Professor of Pediatrics and Cell Biology, University of Massachusetts Medical School
(Presented By: Mary M. Lee, MD)

Acquisition of secondary sexual characteristics and rapid somatic growth are hallmarks of the pubertal transition from childhood to adulthood that culminates in sexual maturity. These physical changes occur in parallel with emotional, behavioral, and cognitive maturational processes. Puberty encompasses both gonadarche (maturation of the testes) and adrenarche (maturation of the adrenal glands). These are temporarily linked but distinct processes that are regulated independently. While the control of adrenarche remains poorly understood, nutritional status, adiposity, familial patterns, ethnic background, and prenatal factors all appear to be modifying influences. For gonadarche, a functioning hypothalamic-pituitary-gonadal (HPG) axis is a prerequisite and can be influenced by a myriad of factors, including genetics, state of health (both physical and psychological), and nutritional status. Recent studies have identified new players in the neuroendocrine regulation of the gonadal axis and revealed novel insights on the interactions of metabolic pathways with the HPG axis.

In males, adrenarche and gonadarche are both associated with an increase in secretion of androgenic steroids, thus their activation promotes overlapping physical changes. In the normal state, acne, body odor, and pubic hair constitute signs of puberty traditionally attributed to adrenal steroids, whereas virilization of the genitalia, deepening of the voice, and facial hair growth typically require the higher concentrations of testosterone secreted by the testes. The distinguishing feature that enables clinicians to discern HPG activation is testicular enlargement, requiring pituitary LH and FSH stimulation. Photographs exemplifying the five stages of puberty are used world-wide to visually assess development of pubic hair and genitalia. Measurement of testicular volume using Prader beads, a set of elliptical beads of increasing sizes, enables an additional and more precise determination of pubertal onset. Recent studies have suggested a secular trend toward earlier onset in girls but data is boys is more limited and the traditional criteria for delineation of normal and abnormal onset of puberty still apply. Boys who enter puberty before the age of 9 years are considered to have early puberty and those who fail to manifest any signs of puberty by 14 years are considered delayed. In addition to timing of pubertal onset, the pace of puberty also needs to be considered in evaluating pubertal disorders. Those boys who progress extremely slowly or who remain at a stage of puberty without further maturation may have dysfunction of the HPG axis. The evaluation and differential of early and late puberty and the implications of these disturbances in pubertal development for adult reproductive health will be discussed.

SYMPOSIUM III - Size Does Matter! Growth in the Juvenile
Roles of Testicular MicroRNAs
Wei Yan, MD, PhD
Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV
(Presented By: Wei Yan, MD, PhD)

MicroRNAs (miRNAs) represent a subclass of small noncoding RNAs that are believed to mainly function as post-transcriptional regulators of gene expression. Genetic ablation of Dicer, a key enzyme required for the production of mature miRNAs, in spermatogonia or Sertoli cells has revealed that Dicer-dependent miRNAs are essential for the development and normal function of these two testicular cell types. Our recent work on selective inactivation of Dicer in pachytene spermatocytes and round spermatids demonstrated that miRNAs were indispensable for the meiotic and haploid phases of spermatogenesis. Using the deep sequencing technology, we have defined the miRNA transcriptome of major testicular cell populations including Sertoli cells, Leydig cells, primitive type A spermatogonia, pachytene spermatocytes, round spermatids and elongated spermatids. Large-scale of expression profiling analyses identified many testis-specific miRNAs. Bioinformatic and reporter assays of miRNA targets of these testis-specific miRNAs revealed that 1) testis-specific miRNAs tend to target testis-specific miRNAs, 2) one miRNA can target multiple mRNAs, and 3) one mRNA can be targeted by numerous miRNAs. The interwoven relationship between miRNAs and their target mRNAs appear to act as a fail-safe mechanism, suggesting important roles of these miRNAs in spermatogenesis. ~20% of the testicular miRNAs are encoded by X chromosome and most of the X-linked miRNAs escape the meiotic sex chromosome inactivation (MSCI) and the post-meiotic sex chromatin (PMSC). This finding represents the first observation of X-linked genes escaping the pan-chromosomal silencing effects, implying possible critical roles of these X-linked miRNAs. Using the pachytene spermatocytes-round spermatids-specific Dicer knockout mice, we have demonstrated that miRNAs were involved in the regulation of translational status of haploid miRNAs by confining those transcripts to the ribonuclear protein particles (RNP) through interactions with the RNA-induced silencing complexes (RISCs). The role of miRNAs in initiation and maintenance of MSCI and PMSC is under extensive investigation in my laboratory. This work is supported by NIH grants (HD048855 and HD050281).
SYMPOSIUM III - Fertility in Adult Life
Sperm and Your Natural Marijuana: Endocannabinoids, Nicotine and Fertility
Lani J. Burkman1,2, Stuti Tambor1, Herb Schuel1, Ellen Pollack1, Rachel Rafferty1, Asad Rizvi1, Fadi Joulaik1,2.
1Dept. of Gyn/Ob, University at Buffalo, NY, 2LifeCell Dx, E-fertility Diagnostics, Buffalo, NY, 3Dept. Physiological Sciences/Anatomical Sciences, University at Buffalo
(Presented By: Lani J. Burkman, PhD)

Objectives: Provide background for understanding endocannabinoids and their receptors within the context of human reproduction. All tissues of the body either synthesize endocannabinoids (ECB) or have receptors for ECB, including sperm. Sperm respond to marijuana, and also have cholinergic receptors which bind to nicotine and cotinine. It is possible that both of these signaling systems could regulate sperm function.

Methods: Semen parameters and/or capacitating sperm functions were assessed for men who were drug-free (donors), and for groups of men who habitually used marijuana or nicotine. All studies utilized CASA evaluation, including Burkman Hyperactivation values (HA). Certain data sets used acrosomal status or sperm binding to the Hemizona pellucida as endpoints. With the donor experiments, sperm were incubated with receptor agonists or antagonists. Statistical findings used P <0.05 as the cut-off.

Results and Conclusions: Early studies revealed that human seminal plasma contains anandamide and two other ethanolamides, consistent with the fact that CB receptors are present in many reproductive tissues of the male. Sperm functions in vitro are altered primarily by CB-1 receptor ligands. Men who used marijuana several times per week had lower seminal volume, but a very high incidence of HA swimming. We proposed that the ECB system may interact with the sperm cholinergic system. In vitro, low and high concentrations of Nicotine+Cotinine (NC) significantly affected sperm swimming patterns and acrosomal membrane changes. Several other compounds which bind to cholinergic receptors produced significant changes in sperm function. Most tobacco users that we tested showed deficient sperm binding to the zona; however, the addition of a CB compound reversed the binding loss. We continue to pursue the use of advanced semen analysis in the clinical setting (LCPredict) — for infertile men who abuse marijuana, nicotine, methamphetamine, etc., whether as walk-in patients or remote patients served uniquely via telemedicine.

SYMPOSIUM III - Fertility in Adult Life
Phosphorylation Events During Sperm Capacitation
Dario Krapf1, Enid Arcelay1, Eva V. Wertheimer1, Archana Sanjay2, Ana M. Salicioni3, Stephen H. Pilder3, Pablo E. Visconti4.
1Department of Vet. and Animal Sci., Univ. of Massachusetts, Amherst, MA; 2Dept. of Anat. and Cell Biol., Temple Univ. School of Med., Philadelphia, PA; 3Presented By: Pablo E. Visconti, PhD)

Mammalian sperm capacitation involves a series of sequential and concomitant processes; some of which take place as soon as the sperm is ejaculated while others arise over a longer period in the female tract or in a medium that supports in vitro capacitation. Signaling events leading to capacitation rely on the activation/deactivation of proteins by phosphorylation. This pathway includes SACY, an atypical bicarbonate-stimulated adenylyl cyclase, and is mediated by protein kinase A (PKA) and the subsequent stimulation of protein tyrosine phosphorylation. Recently, it has been proposed that the capacitation-associated increase in tyrosine phosphorylation is mediated by Src tyrosine kinase. In this presentation, a general overview of this pathway will be presented with particular emphasis in the role of c-Src in the regulation of protein tyrosine phosphorylation on sperm capacitation. In previous studies, the conclusion that c-Src is the tyrosine kinase linking PKA with the capacitation-associated increase in protein tyrosine phosphorylation was based in the observation that Src is present in sperm; in addition, Src kinase family inhibitor SU6656 blocked the capacitation-associated increase in tyrosine phosphorylation. Our results confirm those observations and provide further evidence that these Src inhibitors are also able to prevent PKA phosphorylation upstream of the increase in tyrosine phosphorylation as well as sperm progressive motility and in vitro fertilization. These data together with the observation that sperm from c-Src KO mice have no defects in phosphorylation pathways suggest that the Src pathway in sperm is more complex than previously thought, and that c-Src is unlikely to be the sole link between PKA and the downstream pathways leading to the increase in tyrosine phosphorylation.

LECTURE III: Male Reproductive Tract Function
Bernard Robaire and Mahsa Hamzeh
Departments of Pharmacology and Therapeutics and of Obstetrics and Gynecology, McGill University, Montreal, QC, Canada
(Presented By: Bernard Robaire, PhD)

Although it is well established that the epididymis is critically dependent on the presence of the testis, and that androgens play an essential role in maintaining the integrity and functions of this tissue, removal of the testis, and therefore the source of androgens, does not result in a decrease in androgen receptors (AR) in this tissue. Indeed, administration of androgens to a regressed epididymis results in restoration of many, although not all, of its cellular functions. Our objectives were to first establish the sequence of gene expression responses that occurs in an androgen deprived tissue upon re-administration of the two metabolites of testosterone, dihydrotestosterone (DHT) and estradiol (E2) and then to determine the molecular mechanisms that are triggered when androgen-deprived epididymal cells are exposed to androgens. By 12 h after E2 treatment of animals that had been orchidectomized for eight days, nerve growth factor receptor and S100 calcium binding protein G were two of the most dramatically regulated genes. At the same time point, DHT had caused remarkable responses in endothelin 1, bone morphogenetic protein 4, insulin-like growth factor binding protein 3 and insulin-like growth factor 1 (lgf1). Using pathway analysis, we identified epidermal growth factor and lgf1 as hubs in the regulation of the initiation of androgen action. To determine the potential signaling pathway(s) activated by DHT, we used the caput epididymal cell line (PC-1). We found that DHT did not act via Akt activation but caused an activation of the ERK pathway by 1 min; this was transient and disappeared by 15 min. Interestingly, this transient ERK activation was blocked by the antianandrogen, hydroxyflutamide. In addition, SRC kinase was activated by DHT and was required for ERK activation. We also established that EGFR and IGRF1 were downstream of Src and that they contributed to ERK and CREB phosphorylation. We postulate that this rapid activation of androgen may act ultimately to modulate transcriptional activity of genes regulated by AR in the nucleus. These results provide a better understanding of the molecular mechanisms of the initiation of androgen action in epidymal epithelial cells. Supported by CIHR.

LECTURE IV: Translational Andrology: Bridging Bench and Bedside
Christina Wang and Ronald S. Swerdloff
Division of Endocrinology, Department of Medicine, and the General Clinical Research Center at Harbor-UCLA Medical Center, Los Angeles Biomedical Research Institute, Torrance, CA
(Presented By: Christina Wang, MD)

Traditional research can be broadly defined as the application of innovations in science to advances in medicine and their adoption into clinical practice. In Andrology, scientific breakthroughs and their translation into medical practice may not be as evident as in cancer research where innovative new treatments can potentially cure an otherwise terminal disease. Nevertheless, breakthroughs in Andrology easily translate into major factors in determining the quality of life. Discoveries of Y chromosome micro-deletions have
altered the assessment of the infertile couple. Testicular sperm extraction followed by intracytoplasmic injection of sperm changed the outlook of men with Klinefelter’s Syndrome (KS) who desire to father a child. The success of the treatment with TESA and ICSI for KS men with infertility does not provide insight as to why an extra X chromosome causes disappearance of germ cell in adults, dysfunctional Leydig cells leading to hypogonadism, and susceptibility to a myriad of other clinical manifestations. Our group used a XXY mouse model to show that many of the characteristics in KS patients are manifested in XXY mice. Thus the mouse model provides the means to uncover the genetic or epigenetic mechanisms induced by the extra X chromosome. Understanding these mechanisms may lead to methods to alleviate the problems before the irreversible changes occurred in men. Another example of successful translational accomplishment in Andrology is that current male hormonal contraceptive trials show definitively that suppression of sperm output by exogenous hormone provides efficacious contraception. The concept was developed in animal models and then applied to men. We and others have shown that the principal mechanisms of deprivation of intratesticular hormones include induction of germ cell death, dysfunctional spermatiation and decreased spermatogonia proliferation. Germ cell apoptosis occurred predominantly via the mitochondria pathway through the disturbance of the balance between pro-apoptotic and pro-survival factors from rodents to men. One of the goals of our group is to delineate testis specific regulators of germ cell death that may provide the stimulus to accelerate apoptosis without the administration of exogenous hormones. While at the same time, the need and the acceptability of male contraceptives have been re-evaluated in different regions of the world. The translation of discoveries back to the clinic requires not only the applied feasibility of the discovery but also the development by interested industry partners and the acceptability of the new discovery by the community.

TUESDAY, APRIL 13, 2010
8:00 a.m. – 9:30 a.m.
SYMPOSIUM IV – Aging and Male Reproductive Health

Effects of Aging and Environmental Insults on the Sperm Genome
Andrew J. Wyrobek, PhD

With aging, serum testosterone (T) levels decline gradually and progressively in men, such that an increasing proportion of aging men have T levels below the normal range in younger men. The age-related decline in T levels is associated with alterations in body function (e.g. reduced muscle mass, bone density and sexual function) that are comparable to those due to androgen deficiency in young hypogonadal men that improve with T therapy. Thus, it is hypothesized that the decline T levels contribute to age-associated alterations in body function, sparing terms such as late-onset hypogonadism. In practice, the diagnosis of male hypogonadism is made only in men with clinical manifestations of androgen deficiency and low serum T levels. As so defined, the prevalence of male hypogonadism is made only in men with clinical manifestations of androgen deficiency and low serum T levels. As so defined, the prevalence of male hypogonadism in a community population is ~6-12%, increasing significantly with age. However, there are significant challenges in diagnosing hypogonadism, especially in older men: 1) Manifestations of T deficiency are non-specific and modified by age, co-morbidities and severity of deficiency; 2) T concentrations exhibit considerable variability and may be affected by illness, medications and alterations in SHBG; 3) The T level that is associated with manifestations of androgen deficiency and improvement with T treatment is not clear and differs with the specific manifestation. Furthermore, some outcomes e.g. muscle mass, exhibit a linear dose-response to T treatment without a threshold. Small, short-term controlled trials of T treatment in older men with low-normal to low T levels have demonstrated inconsistent beneficial effects but have had significant limitations. An ongoing NIH multi-center randomized, placebo-controlled trial in older men with symptoms of androgen deficiency and unequivocal consistently low T levels will more definitively assess the short-term effect of T on physical, sexual and cognitive function and vitality. Recently, low T has been associated with important clinical outcomes, e.g. dementia, diabetes, heart disease and mortality. However, clinical benefits and risks (e.g. on prostate cancer) of T treatment in older men remain to be determined in a larger long-term, prospective, randomized controlled trial. Funding Provided by None.

TUESDAY, APRIL 13, 2010
8:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Aging and Male Reproductive Health

The Relationship of Paternal Age to Health and Disease in Offspring
Susan Harlap, MD
New York University School of Medicine
(Presented By: Susan Harlap, MD)

During the past 50 years, both medical professionals and the general public have learned that pregnancies of older mothers carry an increased risk of sub-optimal outcomes. With the goal of a healthy offspring, women have been admonished to complete childbearing before age 35. The contribution of men to reproduction has been ignored for too long, little thought being given to the obvious truth that older mothers tend to have even older partners. Now, there is a new focus on the man; it has become clear that paternal aging contributes not only to women’s pregnancy complications and to birth defects, but also to the short and long term survival of progeny, to malignancies such as leukemia and early-onset prostate and breast cancer, and to complex diseases such as schizophrenia, bipolar disorders and autism. Processes in the aging testis contribute to a variety of inheritable changes in DNA, the best known being the point mutations that accumulate over time and which are analogous, if not identical, to those accumulating in somatic cells. Male aging is also associated with a few types of structural rearrangements of chromosomes in sperm and some in progeny; with alterations in copy number in repetitive DNA; with changes in methyl transferases that may cause alterations in epigenetic marks; and with altered length of telomeres. In addition, transmissible genetic causes of male sub-fecundability will, by delaying parenthood, tend to be passed more often to the offspring of older fathers. De novo point mutations, when detected in progeny, are almost always inherited from fathers; these are found for many different birth defects but each is sporadic, individually rare and unlikely to recur. Certain mutations, however, may confer a growth-promoting selective advantage to the clones of spermatogonial stem cells bearing them, constituting, in one sense, a benign neoplasm. Some of these give rise to specific groups of birth defects and malignancies that show an overwhelmingly strong association with paternal age. Although there may be deleterious consequences for individual offspring associated with paternal aging, most elderly fathers can expect to sire normal healthy offspring. At the population level, the changes associated with paternal ageing may benefit human evolution.

TUESDAY, APRIL 13, 2010
9:45 a.m. – 10:45 a.m.

INTERNATIONAL LECTURE:
Mechanisms of Fertilization – A View Through Gene Manipulated Mice
Masaru Okabe, PhD
Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Japan
(Presented By: Masaru Okabe, PhD)

Recent gene knockout approaches have revealed that many of the factors previously considered to be “important” were largely dispensable in gene knockout animals, while previously unknown factors are emerging. Sperm from five different gene-disrupted mouse lines (calmegin (Clgn), Adam1a, Adam2, Adam3 and Ace) are all known to have defective zona binding ability. Very interestingly, in all of the five lines, the phenotype of impaired
We have previously reported that sperm require IZUMO1, while eggs require sperm binding to zona pellucida was accompanied by the loss of sperm-sperm recognition mechanism will be discussed. During fertilization, the distribution of IZUMO1 is not limited to the equatorial segment of the acrosome-reacted sperm in the equatorial segment membrane, which usually forms an acrosomal sheath, disappears after acrosome reaction in Spesp1-deficient mice. The mechanisms of fertilization will be discussed by observing various kinds of gene-manipulated animals. Funding provided by grants from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

**SYMPOSIUM V - Long-Lasting Sex**

**The Therapeutic Potential of Germ Stem Cells**
Amander Clark, PhD
Department of Molecular Cell and Developmental Biology, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles

(Presented By: Amander Clark, PhD)

Germ cells are responsible for the passage of genetic information between generations. For this reason, the germ line represents one of the most important lineages for survival of man. Germ cell differentiation can be defined in a series of stages. The first stage occurs during fetal life where the germ line differentiates from the epiblast at implantation. These primitive germ cells are called primordial germ cells (PGCs), which undergo considerable nuclear reprogramming to create a pool of PGCs that populate the fetal gonad. Normal differentiation of PGCs is critical because these cells represent the founder cells that give rise to the spermatogonial stem cells of the testis. The second stage of germ cell development is quiescence, in which the spermatogonial stem cells exhibit a slow rate of turnover. The third stage of germ cell differentiation is spermatogenesis, where spermatogonial stem cells are recruited to differentiate into haploid spermatozoa. Compromised differentiation at any of these stages can result in infertility, however compromised differentiation at the PGC stage is the most devastating as this has the potential to render an individual agametic. My laboratory studies PGC development in mouse and humans using embryonic stem cells as a model. The goal of our work is to develop a strategy to differentiate functional PGCs that are capable of contributing to the birth of live healthy and fertile offspring. This technology could be used in the future to treat infertility in humans if starting with induced pluripotent stem cells. Furthermore, we are also defining the molecular mechanisms responsible for PGC differentiation with a specific focus on the role of protein arginine methyltransferases (PRMTs). We will present data demonstrating that PGCs can be reliably differentiated from mouse and human ESCs. We will also show that a gonadal niche is required for PGC maturation. Using human fetal gonads, we will show that PGCs express high levels of PRMT5, and we hypothesize is critical for PGC differentiation. Finally, we will present genetic evidence through stable knockdown of PRMT5 that reveals its role in regulating germ cell-specific gene expression.

**SYMPOSIUM V - Long-Lasting Sex**

**Love and Monogamy: An Endocrine Story**
C. Sue Carter, PhD
Brain Body Center, University of Illinois at Chicago

(Presented By: C. Sue Carter, PhD)

Objectives: The neuroendocrine regulation of social and emotional behavior by mammalian peptides, will be the focus of this talk. Our specific purpose is to examine various functions of oxytocin across the mammalian lifespan. For example, although oxytoicin (aka "piloerin") is widely used in medicine to manipulate the birth process, the consequences for the behavior of either the mother or her child are only now being questioned. Methods: Of particular importance in understanding the behavioral and emotional effects of neuroptides have been studies in socially monogamous prairie voles. Using this rodent model and studying both sexes, the role of oxytocin, and the related peptide vasopressin, in the development of social bonds and parental behavior have been examined. Using measurements of endogenous oxytocin and through the use of exogenous oxytocin, conceptually-related research has also been conducted in humans (including individuals with disorders of social behavior such as autism). Results: Taken together these studies strongly implicate oxytocin and vasopressin in social and emotional behaviors in humans and other mammals. The effects of oxytocin and vasopressin are often sexually-dimorphic, with males particularly sensitive to endocrine or social manipulations in early life. Conclusion: There is increasing evidence that oxytocin and vasopressin are components of systems responsible for sex differences in social and emotional behaviors. The same hormones that regulate birth and sperm transport, also coordinate sexual and social behaviors. In addition, human concepts such as "monogamy" and even "love" have been traced to the functions of these molecules.

Funding provided by NIH (MH 072935; MH 073022)

**SYMPOSIUM V - Long-Lasting Sex**

**Long-Lasting S-E-X (Science, Education, Xenogamy)**
Anna Steinberger, PhD
Professor Emerita, UTMS-Houston (retired)

(Presented By: Anna Steinberger, PhD)

Part I will provide an overview of the development and the importance of SCIENCE, its history from antiquity to the modern period, basic classification of scientific fields, and the value of using accepted scientific methods. Also the concepts of hypothesis, evidence, modeling, and theory will be discussed briefly.

Part II will deal with EDUCATION; its formative effects on the mind, character, and physical ability of an individual in achieving full potential; the process by which society transmits its accumulated knowledge, skills, and values from one generation to the next. It will include also the process by which we learn, various learning modalities, the role of technology in learning; and educational theory, economics, philosophy, and internationalization.

Part III will consider the role of XENOGAMY (crossbreeding) in promoting diversity, enhancement of fitness and hybrid vigor. While it has been accepted that XENOGAMY can be highly beneficial in the plant and animal kingdoms, there are also huge benefits from XENOGAMY between basic and clinical fields, Andrology being no exception!! Scientific findings derived from basic research can have significant impact on clinical investigations and patient care. Vice versa, experiences and observations gained from clinical observations and clinical research often stimulate new directions for basic research that can lead to better understanding of physiologic and pathologic conditions as well as more rational approaches to therapy.

This form of XENOGAMY was a major goal in the establishment of the American Society of Andrology, a Society where basic and clinical investigators, and practicing physicians, could exchange ideas, express opinions and initiate collaborative research.
Sunday, April 11, 2010
11:00 a.m. – 12:30 p.m.
Location: Colonnade Salon

**EPIDIDYMIS / VAS DIFFERENTS / SEMINAL VESICLES**

**Poster #13**
**REGULATION OF PANNEXIN-1 AND PANNEXIN-3 IN THE ADULT RAT EPIDIDYMIS**
Daniel Cyr, PhD¹, Patrick Turmel, BSc², Julie Dufresne, MSc³, Charles Smith, PhD³, Silvia Penuela, PhD³, Dale Laird, PhD³, and Louis Hermo PhD³

¹INRS-Institut Armand-Frappier; ²McGill University; ³University of Montreal; ⁴University of Western Ontario
(Presented By: Daniel Cyr, PhD)

**Poster #14**
**INFLUENCE OF MERCURIC CHLORIDE ON ANTIOXIDANT SYSTEM IN THE TESTIS AND EPIDIDYMIS OF ALBINO RATS**
Venugopal Ramalingam, MSc, MPhil, PhD¹, Suganthy Onerie Arcentreline, MSc, MPhil, MEd¹, Subbarayalu Panneerdoss, MSc, MPhil, PhD² and Viswanadhapalli Suryavathi, MSc, MPhil, PhD²

¹Lecturer; ²Scientist
(Presented By: Venugopal Ramalingam, MSc, MPhil, PhD)

**Poster #15**
**TESTICULAR LUMINAL FLUID FACTORS REGULATE MAPK, JAK/STAT AND NFkB PATHWAYS IN THE INITIAL SEGMENT OF RAT EPIDIDYMIS TO PREVENT CELL APOPTOSIS**
Bingfang Xu, Rana Abdel-Fattah, Ling Yang, Sallie Crenshaw and Barry Hinton
(Presented By: Bingfang Xu)

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**FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT**

**Poster #16**
**PROTEOMIC ANALYSIS OF GUINEA PIG SPERM ACROSOMAL CONTENTS AND HYBRID VESICLE MEMBRANES**
Vincent Arnone¹, Alex Johnson¹, Mariano Buffone, PhD², George Gerton, PhD² and James Foster, PhD²

¹Randolph-Macon College; ²University of Pennsylvania
(Presented By: Vincent Arnone)

**Poster #17**
**THE DIFFERENT DISTRIBUTION OF SMALL RNAs OF SOMATIC AND GERM LINE CELLS IN HUMAN TESTES**
Eiitsu Koh, PhD, MD¹, Ho-su Sin, MS, Kazuhiro Sugimoto, PhD, MD, Yuji Maeda, PhD, MD, Atsumi Yoshida, PhD, MD² and Mikio Namiki, PhD, MD

¹Kanazawa University Graduate School of Medical Science; ²Reproduction Center, Kiba Park Clinic
(Presented By: Eiitsu Koh, PhD, MD)

**Poster #18**
**FILLING THE HOLES IN THE HUMAN GENOME: DISCOVERY OF THE SPERM ACROSOMAL PROTEIN SSMP20**
Nathaly Cormier, MSc¹, John McGlone, PhD² and Daniel Hardy, PhD²

¹Texas Tech University Health Sciences Center; ²Texas Tech University
(Presented By: Nathaly Cormier, MSc)

**Poster #19**
**PROTEIN RESTRICTION IN UTERO COMPROMISES PUBERTY ONSET AND SPERM ENDPOINTS IN ADULT RATS**
Fabiola Toledo, MS¹, Juliana Perobelli, MSc¹, Flavia Pedrosa¹, Carla Fernandez, MS¹, Ana Paula Favareto, MSc² and Wilma Kempinas, PhD²

¹State University of Campinas; ²Sao Paulo State University
(Presented By: Wilma Kempinas, PhD)

**Poster #20**
**INSL3/RXFP2 AND ANDROGEN SIGNALING IN TESTICULAR DESCENT**
Elena Kaftanovskaya, PhD¹, Shu Feng, PhD², Anne Truong, BS², Sukhinder Kuar, MS², Rhea Pereira, BS², Agustin Barbara, BS² and Alexander Agoulnik, PhD²

¹College of Medicine, Florida International University; ²Baylor College of Medicine
(Presented By: Alexander Agoulnik, PhD)

**Poster #21**
**FUNCTIONAL ASSESSMENT OF CRYOPRESERVED BOVINE GONOCYTES: ISOLATED CELLS VS TISSUE**
Zhen Zhang, MD, PhD¹, Xiaojian Wang, PhD², David Galloway, PhD², Sigrid Lehnert, PhD³ and Michael Holland, PhD³

¹Monash Institute of Medical Research; ²CSIRO; ³University of Queensland
(Presented By: Michael Holland, PhD)

**Poster #22**
**EXPRESSION OF C-KIT MRNA AND KIT PROTEIN DIFFERS IN SPERMATOGENIAL STEM CELLS BEFORE AND AFTER DIFFERENTIATION IN MOUSE**
Lei Zhang, Christopher John Haines, MD and Yibing Han, PhD
Dept of OB/G, CUHK
(Presented By: Lei Zhang)

**Poster #23**
**FERTILIZATION DEFECTS IN SPERM FROM TYROSYLPROTEIN SULFOTRANSFERASE 2 (TPST2)-DEFICIENT MICE ARE LINKED WITH ABNORMALITIES IN A DISINTEGRIN AND A METALLOPROTEASE (ADAM) PROTEINS AND NOT ABNORMALITIES IN IZUMO**
Matthew Marcello¹, Weitao Jia, PhD², Julie Leary PhD², Kevin Moore, MD² and Janice Evans, PhD²

¹Johns Hopkins University; ²University of California at Davis; ³Oklahoma Medical Research Foundation
(Presented By: Matthew Marcello)
SPERMATOCYTES / STEREOGENESIS / TESTIS BIOLOGY

Poster #24  AGING CAUSES DIFFERENTIAL GENE EXPRESSION IN THE PACHYTENE SPERMATOCYTES IN THE BROWN NORWAY RAT
Catriona Paul, PhD; Makoto Nagano, PhD; and Bernard Robaire, PhD

Poster #25  ANALYSIS OF HISTONE DEMETHYLASE JMJD2D IN SPERMATOCYTES
Naoki lwamori, PhD, Ming Zhao, PhD, Marvin Meistrich, PhD and Martin Matzuk, MD, PhD

Poster #26  RETINOIC ACID AVAILABILITY DRIVES THE ASYNCHRONOUS INITIATION OF SPERMATOCYTES
Elizabeth Snyder, BS; Christopher Small; Kwan Hee Kim; and Michael Griswold

Poster #27  EFFECT OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) REPLACEMENT ON GONADOTROPIN RELEASING HORMONE-ANTAGONIST (GnRH-A) INDUCED MALE GERM CELL APOPTOSIS IN IGFBP-3 KNOCKOUT MICE
Yue Jia MD, PhD, Hemal Mehta, PhD, Kuk Wha Lee, PhD, Laura J. Cobb, PhD, Yanhe Lue, PhD, Vinicius Atienza, PhD, Penchas Cohen, MD, Ronald S. Svedloff, MD and Christina Wang, MD

Poster #28  CHARACTERIZATION OF A NOVEL TEKTIN MEMBER, TEKT5 IN MOUSE SPERM
Wenlei Cao MD, PhD, Takashi Ijiri, PhD, Andy Huang and George Gerton, PhD

Poster #29  TESTICULAR HYPERTHERMIA DECREASES RNA HELICASE DDX4 EXPRESSION IN APOPTOTIC GERM CELLS
YanHe Lue, MD, Katherine Fong, Brian Li, Jessica Kwan, David Skarbrevik, Ashley Han, Ronald Svedloff, MD and Christina Wang, MD

Poster #30  TRANSCRIPTIONAL CONTROL OF THE ROUND SPERMATID-SPECIFIC ACRV1 GENE BY TAR DNA BINDING PROTEIN OF 43 KD (TDP-43)
A.S. Lalmansingh, C. Urekar and P.P. Reddi

Poster #31  ANALYSIS OF INTERCELLULAR BRIDGE PROTEINS
Tokuko Iwamori, PhD, Naoki Iwamori, PhD, Lang Ma, MA, Mark Edson, BS, Michael Greenbaum, MD, PhD and Martin Matzuk, MD, PhD

Poster #32  MOUSE TESTICULAR DAMAGE IN CONTINUOUS AND INTERMITTENT HYPOXIA
Eduardo Bustos-Obregon, MSc, MD and Alvaro Vargas, DVM, MSc

Poster #33  BENEFICIAL EFFECTS OF QUERCETIN ON SPERM PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC MALE RATS
Arash Khaki, DVM-PhD

Poster #34  ANALYSIS OF MICRONRNA EXPRESSION IN THE PREPUBERTAL TESTIS
Gregory Buchold, PhD, Hufeng Zhu, BS; Cristian Coarfa, BS; Preethi Gunaratne, PhD and Martin Matzuk, MD, PhD

Poster #35  THE DEVELOPMENTAL EXPRESSION OF ACRV1/ACRV1 IN HUMAN AND MICE
Alfa Tang, PhD, Xiaxia Yan Master, Ruiying Diao Master, Zhou Yu Master, Liang Sun Master, Yaoting Gui, PhD and Zhiming Cai, PhD

Poster #36  RAT SPERMATOCYTES DAMAGE IN INTERMITTENT HYPOBARIC HYPOXIA AND THE PROTECTIVE ROLE OF MELATONIN: CAUDA EPIDIDYMAL SPERMATOZOA
Eduardo Bustos-Obregon, MSc, MD and Rodrigo Castro, DVM, MSc

(Presented By: Catriona Paul, PhD)

(Presented By: Naoki lwamori, PhD)

(Presented By: Elizabeth Snyder, BS)

(Presented By: Naoki lwamori, PhD)

(Presented By: Elizabeth Snyder, BS)

(Presented By: Yue Jia, MD, PhD)

(Presented By: Wenlei Cao, MD, PhD)

(Presented By: Yue Jia, MD, PhD)

(Presented By: Aifa Jang, PhD)

(Presented By: Avin Lalmansingh, PhD)

(Presented By: Tomoko lwamori, PhD)

(Presented By: Eduardo Bustos-Obregon, MSc, MD)

(Presented By: Arash Khaki, DVM-PhD)

(Presented By: Gregory Buchold, PhD)

(Presented By: Alfa Tang, PhD)

(Presented By: Eduardo Bustos-Obregon, MSc, MD)
**Poster #37**

**ROLE OF GHRELIN AND LEPTIN IN MALE REPRODUCTION**

Carolina Jorgez, PhD¹, Shannon Whirledge, PhD², Aysegul Sahin, BS³, Roy Smith, PhD³ and Dolores Lamb, PhD⁴

¹BCM Scott Department of Urology; ²BCM Molecular and Cellular Biology Department; ³BCM Huffington Center on Aging

(Presented By: Carolina Jorgez PhD)

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**Poster #38**

**ELMO1 FUNCTIONS IN SERTOLI CELL-MEDIATED APOPTOTIC GERM CELL PHAGOCYTOSIS AND HOMEOSTASIS IN THE TESTES**

Michael Elliott, PhD, Daeho Park, PhD, Robin Woodson, Shuqiu Zheng, PhD, Michael Reardon, Kodi Ravichandran, PhD and Jeffrey Lysiak, PhD

University of Virginia

(Presented By: Michael Elliott, PhD)

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**GENETICS**

**Poster #39**

**A MOLECULAR KARYOTYPE REVEALS NEW CANDIDATE GENE DEFECTS ASSOCIATED WITH COMBINED HYPOSPADIAS AND CRYPTORCHIDISM**

Shaye Lewis, PhD, Mounia Louet, PhD, Carolina Jorgez, PhD, Shuo Han, BS and Dolores Lamb, PhD

Baylor College of Medicine

(Presented By: Shaye Lewis, PhD)

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**Poster #40**

**CDNA MUTATIONS IN ZPB1 ASSOCIATED WITH TERATOZOOSPERMIA IN INFERTILE MEN**

Alexander Yatsenko, MD, Ph.D¹, Anghumoy Roy, MD, Ph.D², Paola Arias-Mendoza, BS³, Ruihong Chen, BS³, Lata Murthy, BS³, Dolores Lamb, Ph.D³ and Martin Matzuk, MD, Ph.D¹

¹Baylor College of Medicine; ²BCM

(Presented By: Alexander Yatsenko, MD, PhD)

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**Poster #41**

**CDNA MUTATIONS IN STYX ASSOCIATED WITH OLIGOZOOSPERMIA IN INFERTILE MEN**

Derek O’Neil, BS, Alexander N. Yatsenko, MD, PhD, Lata J. Murthy, BS, Dolores J. Lamb, PhD and Martin M. Matzuk, MD, PhD

Baylor College of Medicine

(Presented By: Derek O’Neil, BS)

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**Poster #42**

**CLONE AND ANALYSIS OF THE HOMOLOGOUS SEQUENCES OF HUMAN TESTIS DEVELOPMENT RELATED GENE 1 IN DIFFERENT SPECIES**

Tang Yuxin, MD, Wen Jiaming, PhD, Jiang Xianzhen, Chen Houyang, MD, Yang Jianfu, PhD and Liu Zhizhong

(Presented By: Tang Yuxin MD)

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**Poster #43**

**NOVEL PROTIMINE2 (PRM2) MUTATION AND TRANSITIONAL PROTEIN (TP) GENE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) AFFECT SPERM DNA INTEGRITY AND MOTILITY IN INFERTILE MEN**

Sundararajan Venkatesh, Monis Shamsi, Manoj Kumar, Mukesh Tanwar, Rajeev Kumar, Narmada Gupta and Rima Dada

(Presented By: Sundararajan Venkatesh)

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**Poster #44**

**MEIOTIC SEGREGATION PATTERN AND DNA Fragmentation LEVELS IN SPERMATOZOA OF SIX DIFFERENT RECIPROCAL CHROMOSOMAL TRANSLATION CARRIERS WITH REPRODUCTIVE FAILURE**

Marta Olszewska, MSc¹, Monika Fraczek, PhD², Nataliya Huleyuk, PhD³, Ewa Wiland, PhD³, Barbara Panasiuk, PhD³, Alina Midro, PhD, MD³ and Maciej Kurpisz, PhD, MD³

¹Institute of Human Genetics Polish Academy of Sciences; ²Institute of Human Genetics Polish Academy of Sciences, ul. Strzeszynska 32, 60-479 Poznan, Poland; ³Institute of Hereditary Pathology of Academy of Medical Sciences, Ukraine, 79000, L'viv, Lysenko Str. 31a

(Presented By: Marta Olszewska, MSc)

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**Poster #45**

**DIFFERENCES BETWEEN EXPRESSION PROFILES (EP) FROM FRESH SPERM SAMPLES (FHSS) THAT ACHIEVED PREGNANCY VS THOSE UNABLE UNDERGOING AN INTRA CYTOPLASMIC SPERM INJECTION (ICSI) PROCEDURE DISAPPEARED WHEN SPERM IS PREVIOUSLY FROZEN (FRSS)**

Sandra Garcia-Herrero¹, Laura Romany, PhD¹, Manuel Muñoz, MD², Nicolas Garrido, PhD², José-Antonio Martinez-Conejero, PhD¹ and Marcos Meseguer, PhD¹

¹IVI Valencia; ²IVI Alicante

(Presented By: Sandra Garcia-Herrero)

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**Poster #46**

**Y CHROMOSOME MICROLETIONS AND PARTIAL DELETIONS OF THE AZFc REGION IN NORTH INDIAN INFERTILE MEN**

Madhukar Dama, Master of Veterinary Sciences¹, Ahmad Kaleem, PhD², Abbas Mahdi, PhD² and Rajender Singh, PhD²

¹Division of Endocrinology, Central Drug Research Institute, Lucknow, India; ²Department of Biochemistry, CSMMU, Lucknow, India

(Presented By: Madhukar Dama, Master of Veterinary Sciences)

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**Poster #47**

**ANALYSIS AND SIGNIFICANCE OF Y CHROMOSOME TRANSSCRIPTS IN HUMAN EjACULATED SPERM FROM NORMOZOOSPERMIC DONORS: COMPARISON WITH SPERMATOZOA FROM INFERTILE MEN**

Madhukar Dama, MVS¹, Ahmad Kaleem, PhD², Abbas Mahdi, PhD² and Rajender Singh, PhD²

¹Endocrinology, CDRI, Lucknow, India; ²Department of Biochemistry, CSMMU, Lucknow, India

(Presented By: Rajender Singh, PhD)
Identifying Copy Number Variants Associated to Genitourinary Birth Defects and Characterization of Novel Key Genes in Human Genital Development
Shue Han, Mounia Tannour-Louet, PhD, Sean Corbett, MD and Dolores Lamb, PhD
Department of Urology, Baylor College of Medicine
(Presented By: Shue Han)

Identification of Copy Number Variants Associated to Genitourinary Birth Defects and Characterization of Novel Key Genes in Human Genital Development
Shue Han, Mounia Tannour-Louet, PhD, Sean Corbett, MD and Dolores Lamb, PhD
Department of Urology, Baylor College of Medicine
(Presented By: Shue Han)

Male Sexual Function

Inflammation May Play an Important Role in the Erectile Dysfunction Associated with Type 1 Diabetes
Donghua Xie, MD, PhD and Craig Donatucci, MD
Duke University Medical Center
(Presented By: Donghua Xie, MD, PhD)

Correlation of Serum Testosterone Levels in Men Presenting with Erectile Dysfunction Using Different Validated Questionnaires
Osama Mohamed, MD, Kumaran Sathyamorthy, MD, MPH, Larry Lipshultz, MD and Mohit Khera, MD, MPH
Baylor College of Medicine
(Presented By: Osama Mohamed, MD)

Effect of Mucuna Pruriens (Linn.) on Sexual Behavior and Sperm Parameters in Streptozotocin Induced Diabetic Male Rats
Seppan Prakash, PhD, Sekar Suresh, MSc and Elumalai Prithviraj, MSc
University of Madras
(Presented By: Seppan Prakash, PhD)

Weight Loss Improves Sexual and Lower Urinary Tract Function in Obese Diabetic Men: Effects of Energy Restriction, Nutritional Quality and Time Course
Gary Wittert, MBch, MD, FRACP1, Cynthia Plantadosi, PhD1, Rae Duncan, MBBS2, Stephen Worthley, MBBS, PhD1, Alicia Jenkins, MBBS, PhD1, Kylie Lange, MSc1, Manny Noakes, PhD1, Matthew Worthley, MBBS, PhD1 and Khoo Joan, MBBS2
1University of Adelaide; 2The Newcastle upon Tyne Hospitals; University of Melbourne; CSIRO Human Nutrition, Adelaide; Changi General Hospital, Singapore
(Presented By: Gary Wittert, MBch, MD, FRACP)

Salvage Penile Curvature Correction Surgery
Cheng-Hsing Hsieh, MD1, Heng-Shuen Chen, MD, PhD2, Wen-Yuan Lee, MD2, Kuo-Liang Chen, MD2, Chao-Hsiang Chang, MD3 and Geng-Long Hsu, MD3
1Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, Taiwan; 2Department of Family Medicine and Department of Medical Informatics, National Taiwan University, Taipei, Taiwan; 3Microsurgical Potency Reconstruction and Research Center, Department of Urology, China Medical University and Hospital, Taichung, Taiwan
(Presented By: Geng-Long Hsu, MD, FRACP)

Salvaging Penile Venous Stripping Surgery
Geng-Long Hsu, MD1, Heng-Shuen Chen, MD, PhD2, Cheng-Hsing Hsieh, MD3, Wen-Yuan Lee, MD1, Kuo-Liang Chen, MD1 and Chao-Hsiang Chang, MD1
1Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, Taiwan; 2Department of Family Medicine and Department of Medical Informatics, National Taiwan University, Taipei, Taiwan; 3Microsurgical Potency Reconstruction and Research Center, Department of Urology, China Medical University and Hospital, Taichung, Taiwan
(Presented By: Geng-Long Hsu, MD, FRACP)

Clinical Characteristics of the Patients with Lifelong Premature Ejaculation of Less Than 1 Minute IELT
Joon Yong Kim, Professor, MD, Byung Moo Philip Kim and Si Jin Paul Kim
Philip and Paul Medical Institute
(Presented By: Joon Yong Kim, Professor, MD)

Salvaging Penile Venous Stripping Surgery
Geng-Long Hsu, MD1, Heng-Shuen Chen, MD, PhD2, Cheng-Hsing Hsieh, MD3, Wen-Yuan Lee, MD1, Kuo-Liang Chen, MD1 and Chao-Hsiang Chang, MD1
1Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, Taiwan; 2Department of Family Medicine and Department of Medical Informatics, National Taiwan University, Taipei, Taiwan; 3Microsurgical Potency Reconstruction and Research Center, Department of Urology, China Medical University and Hospital, Taichung, Taiwan
(Presented By: Geng-Long Hsu, MD, FRACP)

Chronic Use of a Short-Acting PDE-5 Inhibitor to Restore Erection and Ejaculation Before IVF in a Case with Acquired Hypogonadism and Hyperprolactinemia
Juan Correa-Perez, PhD, Andrea Drury, BS and Ronald Wilbois, MD
Infertility and IVF Center
(Presented By: Juan Correa-Perez, PhD)

New Penile Augmentation Surgery Technique by Injecting Micronized Human Cell-Free Dermal Tissues into Subcutaneous Tissue
Joon Yong Kim, Professor, MD, Byung Moo Philip Kim and Si Jin Paul Kim
Philip and Paul Medical Institute
(Presented By: Joon Yong Kim, Professor, MD)

Penile Veins Are the Determining Contributor for Erection: The Hemodynamic Evidence from the Study in Defrosted Human Cadavers
Geng-Long Hsu, MD1, Yi-Ping Huang, PhD2, Mang-Hung Tsai, PhD3, Kuo-Liang Chen, MD3, Chao-Hsiang Chang, MD3 and His-Chin Wu, MD3
1China Medical University; 2China Medical University Hospital
(Presented By: Geng-Long Hsu, MD)
Poster #59  
CABYR, A CANCER-TESTIS ANTIGEN EXPRESSED IN HUMAN LUNG CANCERS – POTENTIAL BIOMARKER FOR LUNG CANCER  
Panneerdoss Subbarayalu, PhD¹; Arabinda Mandal, PhD²; Suryavathi Viswanadhapalli, PhD³; Yuan Liu, PhD⁴; Jagathpala Shetty, PhD⁵; Olga Chertihin, MS¹; David Jones, MD⁶; Mark Jameson, MD, PhD⁷; Henry Frierson, MD⁸; Charles Flickinger, MD⁹ and John Herr, PhD¹  
¹Department of Cell Biology; ²Department of Surgery; ³Department of Otolaryngology - Head and Neck Surgery; ⁴Department of Pathology  
(Presented By: Panneerdoss Subbarayalu, PhD)  

Poster #60  
CAG AND GGN REPEAT LENGTHS AND CAG/GGN HAPLOTYPES VARIATION IN ANDROGEN RECEPTOR GENE AND PROSTATE CARCINOMA IN NIGERIA MEN  
Oluyemi Akinloye, PhD¹; Joerg Gromoll, PhD² and Manuela Simoni, MD³  
¹Reproductive and Molecular Endocrinology Research Laboratory, Department of Clinical Biochemistry, College of Health Sciences, Ladoke Akintola University of Technology; ²Centre for Reproductive Medicine and Andrology of the University of Muenster, Germany; ³University of Modena and Reggio Emilia, Dept. of Medicine, Endocrinology, Metabolism and Geriatrics; Via Giardini 1355 1·41126 MODENA, Italy  
(Presented By: Oluyemi Akinloye, PhD)  

Poster #61  
APOPTOTIC AND PROLIFERATIVE ACTIVITIES IN GERBIL PROSTATE ON CANCER CHEMICAL INDUCTION MODEL AFTER VASECTOMY  
Sergio Pereria, PhD¹; Otavio Martins, PhD²; Patricia Pinheiro, PhD³; Raquel Domenicioni, PhD³; Marcelo Martinez, PhD³; Francisco Martinez, PhD³ and Wilson Melo Jr., PhD³  
¹Department of Anatomy - Bioscience Institute - UNESP; ²Department of Anatomy – IBB – UNESP; ³Department of Morphology and Pathology  
(Presented By: Sergio Pereria, PhD)  

Poster #62  
MAST CELL IN THE PROSTATE VENTRAL LOBE, TESTIS AND EPIDIDYMIS OF UCHB RATS (10% V/V ETHANOL VOLUNTARY INTAKE)  
Francisco Martinez, Dr¹; Leonardo Mendes, Magister²; Luiz Chuffa, Magister¹; João Amorim, Magister¹; Giovana Teixeira, Magister¹; Beatriz Fioruci, Magister²; Tatiana Pimentel, Magister¹; Wilson Júnior, Dr¹; Carlos Padovani, Dr¹; Marcelo Martinez, Dr³; Patricia Pinheiro, Dr¹ and Sônia Oliani, Dr¹  
¹UNESP – Univ Estadual Paulista; ²UNICAMP; ³UFSCar  
(Presented By: Francisco Martinez, Dr)  

Poster #63  
ARE PROSTATE BIOPSIES MANDATORY IN PATIENTS WITH ELEVATED SERUM PROSTATE-SPECIFIC ANTIGEN LEVEL TOGETHER WITH PROSTATITIS OR URINARY TRACT INFECTION?  
Xiao Gu, MD, PhD¹; Jin Yang, MD²; Tao Gu, MD, PhD² and Zhao Hong, MD, PhD³  
¹University of Oklahoma; ²Affiliated Hospital of Yangzhou University; ³State University of New York at Buffalo; ⁴University of Louisville  
(Presented By: Xiao Gu, MD, PhD)  

Poster #64  
PROSTATE CYSTADENOMA PRESENTING WITH OBSTRUCTIVE AZOSPERMIA  
Ashraf Mosharafa, MD¹; Yasser Ragab, MD² and Mohammed Torky, MD²  
Cairo University  
(Presented By: Ashraf Mosharafa MD)
Monday, April 12, 2010
11:00 a.m. - 12:30 p.m.
Location: Colonnade Salon

OTHER

Poster #65
PROTEOMIC ANALYSIS OF HUMAN SPERM NUCLEAR PROTEINS
Eddy Rijntjes, PhD1, Jennifer Maselli, BSc1, Barbara Hales, PhD1, Peter Chan, MD2 and Bernard Robaire, PhD1
1McGill University, Department of Pharmacology and Therapeutics, Montreal, QC, Canada; 2McGill University Heath Centre, Department of Urology, Montreal, QC, Canada
(Presented By: Eddy Rijntjes, PhD)

Poster #66
IDENTIFICATION OF THE MAJOR PROTEINS OF RAM SEMINAL PLASMA USING A PROTEOMIC APPROACH
Carlos Souza, PhD1, João Rego, BSc in Animal Science1, Fábio Nogueira, MSc in Biochemistry2, José Oliveira, PhD in Biochemistry3, Gilberto Domont, PhD in Chemistry2, Diones Santos, PhD in Animal Science2 and Arlindo Moura, PhD in Animal Reproduction3
1Federal University of Ceará; 2Department of Animal Science – Federal University of Ceará; 3Institute of Chemistry – Federal University of Rio de Janeiro; 4Department of Biochemistry – Federal University of Ceará; 5Brazilian Agriculture Research Center
(Presented By: Carlos Souza, PhD)

Poster #67
ADVANCES IN UNDERSTANDING OF THE PENILE ANATOMY IN HUMAN BEINGS: A BRIEF OVERVIEW AND EVIDENCE OF MAMMALIAN EVOLUTION
Geng-Long Hsu, MD
China Medical University
(Presented By: Geng-Long Hsu, MD)

Sperm Function / Semen Analysis

Poster #68
IMPLICATION OF STORE-OPERATED CALCIUM CHANNELS ON P32 APPEARANCE AND SP32 ACTIVITY DURING THE ACROSOME REACTION IN BOAR SPERM
Christian Lessard, MSc1, Margaux Claverie2 and Janice L. Bailey, PhD1
1Laval University; 2UT La Rochelle
(Presented By: Christian Lessard, MSc)

Poster #69
CAPACITATION INCREASES MITOCHONDRIAL MEMBRANE POTENTIAL IN BOAR SPERM
Gianluca Paventi, PhD1, Lessard Christian2, Fasolino Giuliana2, Passarella Salvatore, Professor2 and Bailey Janice L., Professor2
1Dipartimento Scienze Animali, Vegetali, e dell’Ambiente, Università del Molise, via de Sanctis, 86100 Campobasso – Italy; 2Centre de Recherche en Biologie de la Reproduction, Département des sciences animales, Université Laval, Quebec City, Quebec, Canada G1V 0A6; 3Dipartimento Scienze per la Salute, Università del Molise, via de Sanctis, 86100 Campobasso – Italy
(Presented By: Gianluca Paventi, PhD)

Poster #70
THE ASSOCIATION OF AGING, OXIDATIVE STRESS AND DNA INTEGRITY IN HUMAN SPERMATOZOA
Edna Tirado, PhD1, Michele Marquette, PhD2, Joseph Musto, PhD2 and Benjamin Leader, MD, PhD2
1ReproSource Inc; 2UTMB
(Presented By: Edna Tirado, PhD)

Poster #71
SEMEN BACTERIAL INFECTION IN HOLSTEIN BULLS INCREASES THE RATE OF SPERM DNA FRAGMENTATION BUT THIS DELETERIOUS EFFECT CAN BE CONTROLLED USING QUINOLONES
Clara Gonzalez, BS1, Roy Rosa, PhD1, Carmen López-Fernández, PhD1, José L. Fernández, MD1, Michael E. Kjelland, PhD2, Juan F. Moreno, BS1 and Jaime Gosámez, PhD1
1Departamento de Biología-UAM; 2Sexing Technologies, Navasota, TX
(Presented By: Clara Gonzalez, BS)

Poster #72
DNA FRAGMENTATION DETERMINED BY SPERM CHROMATIN DISPERSION TEST (HALOSPERM KIT) IS ASSOCIATED WITH DECREASED MOTILITY AND AN ABNORMAL TOTAL MOTILE COUNT IN MEN OF INFERTILE COUPLES
Burkitt Jensen, MD1, Michael Reed, PhD1, Peace Ezeh, MT2, Amanda Hamic, BS1, Lee Caperton, MD1, Jim Thompson, MD1 and Wayne Kuang, MD1
1Division of Urology, The University of New Mexico, Southwest Fertility Center for Men; 2Center for Reproductive Medicine of New Mexico, Albuquerque, New Mexico; 3Southwest Fertility Center for Men
(Presented By: Burkitt Jensen MD)

Poster #73
ACTIVITY OF THE NA,K-ATPASE α4 ISOFORM IS IMPORTANT FOR MOTILITY AND TO MAINTAIN MEMBRANE POTENTIAL, INTRACELLULAR CA2+ AND PH IN RAT SPERMATOZOA
Tamara Jimenez, Graduate Student, Gladis Sánchez, Research Associate, Eva Wertheimer, graduate Student and Gustavo Blanco, MD, PhD
University of Kansas Medical Center
(Presented By: Gustavo Blanco, MD, PhD)

Poster #74
COMPARISON OF LOW AND HIGH DENSITY SPERM SUBPOPULATIONS IN BOVINE
Olivier D’Amours, MSc, Nancy Allard, Gilles Frenette, BSc, Marlène Fortier, MSc, Pierre Leclerc, PhD and Robert Sullivan, PhD
Université Laval, Centre de recherche en biologie de la reproduction
(Presented By: Olivier D’Amours, MSc)
Poster #75  DIRECT COMPARISON OF TWO PROCESSING TECHNIQUES ON SPERM DNA INTEGRITY
Dennis Marchesi, MS', Hannah Biederman, BS', Hual Feng, PhD' and Avner Hershlag, MD'  
'Center for Human Reproduction, North Shore-LIJ Health System; "Duke University  
(Presented By: Dennis Marchesi, MS)

Poster #76  CONCEIVEASE™, A NOVEL NON-SPERMICIDAL LUBRICANT, MAINTAINS SPERM MOTILITY AND CAN BE SAFELY USED FOR REPRODUCTIVE ISSUES
Kush Patel, MD, Sharon DeWitt, BS, Arthur Caire, MD, Mathew Oommen, MD, Anuj Sharma, Suresh Sikka, PhD and Wayne Hellstrom, MD, FACS  
(Presented By: Kush Patel, MD)

Poster #77  SIMULTANEOUS ANALYSIS OF DNA FRAGMENTATION AND 8-OXOGUANINE IN HUMAN SPERM
Rebeca Santiso, PhD', Maria Tamayo, PhD', Jaime Gosálvex, PhD', Marcos Meseguer, PhD', Nicolás Garrido, PhD' and José Luis Fernández, MD, PhD'  
'INIBIC – Complejo Hospitalario Universitario A Coruña, Unidad de Genética; "Unidad de Genética, Facultad de Biología, Universidad Autónoma de Madrid, Spain; "IVI Valencia, Universidad de Valencia, Spain  
(Presented By: Rebeca Santiso, PhD)

Poster #78  MODIFICATION OF SPERM DNA FRAGMENTATION PARAMETERS AFTER XY SPERM SEX SORTING IN BOVINE
Michael E. Kjelland, PhD', Miguel A. Ramírez, BS', Carmen López-Fernández, PhD', Francisco Crespo, PhD', Kenneth M. Evans', Juan F. Moreno, BS' and Jaime Gosálvex, PhD'  
'Sexing Technologies, Navasota, TX; "Departamento de Biología, Universidad de Genética, Universidad Autónoma de Madrid (UAM); 20849-Madrid, España; "Centro Militar de Cria Caballar de Avila, (FESCCR-Ministerio de Defensa)  
(Presented By: Michael E. Kjelland, PhD)

Poster #79  DIFFERENTIAL MODIFICATIONS OF HUMAN SPERM PEROXIREDOXIN 6 BY REACTIVE OXYGEN SPECIES
Stefan Patrascu, BSc and Cristian O'Flaherty, DVM, PhD  
Urology Research Laboratory, Surgery Dept. McGill University-MUHC  
(Presented By: Cristian O'Flaherty, DVM, PhD)

Poster #80  MOTILE HUMAN SPERMS IN A MICROFLUID DEVICE — A NOVEL TREADMILL FOR COUNTING AND SORTING SPERMES
Vincent F.S. Tsai, MD, MBmEng', Ju-Ton Hsieh, MD', Hong-Chiang Chang, MD', Andrew M. Wo, PhD', Yu-An Chen, Master', Zi-Wei Huang, Master' and Fu-Shan Jaw, Ph D'  
'Tenchen Medical Group, Institute Biomedical Engineering of National Taiwan University; "National Taiwan University Hospital; "Institute of Applied Mechanics, National Taiwan University; "Institute of Biomedical Engineering, National Taiwan University  
(Presented By: Vincent F.S. Tsai, MD, MBmEng)

Poster #81  SPERM PROTECTION BY MILK: BINDING OF BOAR BINDER OF SPERM1 (BSP1) TO MILK PROTEINS
Marie-France Lusignan, MSc and Puttaswamy Manjunath, PhD  
University of Montreal  
(Presented By: Marie-France Lusignan, MSc)

Poster #82  SMALL VARIATIONS IN CRUCIAL STEPS OF TUNEL ASSAY COUPLED TO FLOW CYTOMETRY GREATLY AFFECT MEASURES OF SPERM DNA FRAGMENTATION
Monica Muratori, PhD', Lara Tamburrino, PhD, student', Antonietta Costantino, PhD', Sara Marchiani, PhD', Claudia Giachini, PhD, student', Ilaria Laface, PhD, student', Maria C. Meriggiola, MD', Gianni Forti, MD' and Elisabetta Baldi, PhD'  
'Dep. of Clinical Physiopathology, Sexual Medicine and Andrology Unit, University of Florence; "Department of Obstetrics and Gynecology, Center for Reproductive Health, University of Bologna  
(Presented By: Monica Muratori, PhD)

Poster #83  SUMO-1YLATION OF HUMAN SPERMATOZOA AND ITS RELATION WITH SEMEN QUALITY
Sara Marchiani, PhD', Lara Tamburrino, PhD, student', Lucia Gualano, student in Biotechnology', Daniele Nosi, researcher', Valentina Sarli, student in Biotechnology', Gianni Forti, MD, full professor in Endocrinology', Elisabetta Baldi, associate professor' and Monica Muratori, researcher'  
'Dep. of Clinical Physiopathology, Sexual Medicine and Andrology Unit, University of Florence; "Dept. of Anatomy and Histology, University of Florence  
(Presented By: Sara Marchiani, PhD)

Poster #84  LIGAND BINDING PROPERTIES OF A RECOMBINANT MURINE BINDER OF SPERM HOMOLOG (BSPH1)
Geneviève Plante and Puttaswamy Manjunath, PhD  
Maisonneuve-Rosemont Hospital Research Centre  
(Presented By: Geneviève Plante)

Poster #85  THE TEMPERATURE OF CENTRIFUGATION IS IMPORTANT DURING SPERM INTRA-UTERINE INSEMINATION (IUI) PREPARATION I. KINEMATICAL
Elizabeth Elfano, Manuel Lomas, David Pitts, Rossana Cromwell, George Grunert and Wan-Song Wun  
OGA  
(Presented By: Elizabeth Elfano)
Poster #86

**THE TEMPERATURE OF CENTRIFUGATION IS IMPORTANT DURING SPERM INTRA-UTERINE INSEMINATION (IUI) PREPARATION. II. FUNCTIONAL EXAMINATIONS**

Elizabeth Elefano, Manuel Lomas, David Pitts, Cherie Morgan, Armando Mejia and Wan-Song Wun

OGA

(Presented By: Elizabeth Elefano)

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Poster #87

**GENES INVOLVED IN REPRODUCTIVE PROCESSES (RP) AMONG THOSE GENES DIFFERENTIALLY EXPRESSED (GDE) FROM INFERTILE MALES’ (IM) SPERM SAMPLES (SS) UNDERGOING INTRA CYTOPLASMIC SPERM INJECTION (ICSI) ACHIEVING OR NOT PREGNANCY**

Sandra García-Herrero¹, Laura Romany, PhD¹, Nicolas Garrido, PhD¹, Jose-Antonio Martinez-Conejero, PhD¹, Manuel Muñoz, MD² and Marcos Meseguer, PhD¹

¹IVI Valencia; ²IVI Alicante

(Presented By: Sandra Garcia-Herrero)

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Poster #88

**THE INTAKE OF A SYMPATHOMIMETIC DRUG COMPENSATES FOR THE SEMINAL EMISSION DYSFUNCTION AND SEVERE OLIGOZOOSPERMIA CAUSED BY THE USE OF A SNRI ANTIDEPRESSANT PRESCRIBED FOR GENERALIZED ANXIETY DISORDER/PERIPHERAL NEUROPATHIC PAIN**

Juan Correa-Perez, PhD¹, Pedro Beauchamp, MD², Andrea Drury, BS¹ and Ronald Wilbois, MD²

¹Infertility & IVF Center; ²Puerto Rico Fertility Center

(Presented By: Juan Correa-Perez, PhD)

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Poster #89

**ASSESSMENT OF COMPREHENSIVE TRAINING AND ONGOING QUALITY ASSURANCE FOR TYGERBERG STRICT MORPHOLOGY: A RETURN TO PREDICTIVE VALUE?**

Michael Klug, BS, Jolene Fredrickson, MS, Anthony Krenik, BS and Dean Morbeck, PhD

Mayo Clinic

(Presented By: Michael Klug, BS)

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Poster #90

**HUMAN SEMEN ELECTRICAL IMPEDANCE SPECTRUM UNDER MULTIPLE FREQUENCIES – AN IMPORTANT CHARACTERISTIC FOR BIOLOGICAL MATERIAL AND ITS POTENTIAL FOR COUNTING SPERM**

Vincent F.S. Tsai, MD, MBmEng¹, Andrew M. Wo, PhD², Yu-An Chen, Master², Tzi-Wei Huang, Master², Hong-Chiang Chang, MD³, Juton Haieh, MD³, Allen Y.H. Lai, Master², Fu-Shan Jaw, PhD⁴ and Yi-Hsuan Su, Master⁴

¹Tenchen Medical Group, Institute of Biomedical Engineering of National Taiwan University; ²Institute of Applied Mechanics, National Taiwan University; ³National Taiwan University Hospital; ⁴Institute of Biomedical Engineering, National Taiwan University

(Presented By: Vincent FS Tsai MD, MBmEng)

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Poster #91

**METABOLIC PATHWAYS (MP) AFFECTED AMONG GENES DIFFERENTIALLY EXPRESSED (GDE) FROM INFERTILE MALE’S (IM) SPERM SAMPLES (SS) UNDERGOING INTRAUTERINE INSEMINATION (IUI) ACHIEVING OR NOT PREGNANCY**

Marcos Meseguer, PhD¹, Sandra García-Herrero, PhD², Elena Selles, PhD², Sara Fortuño, MD², Jose-Antonio Martinez-Conejero, PhD³ and Nicolas Garrido, PhD²

¹IVI Valencia, Spain; ²IVI Alicante, Spain; ³GENOMIX, Spain

(Presented By: Marcos Meseguer, PhD)

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**ANDROGENS / ENDOCRINOLOGY**

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Poster #92

**ABNORMAL PATTERNS OF INHIBIN B AND ANDROGEN RECEPTOR IMMUNOEXPRESSION IN SERTOLI CELLS OF SENESCENT MEN AND CHRYPTORCHID PATIENTS**

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(Presented By: Javier Regadera, MD, PhD)

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Poster #93

**EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON DEPRESSIVE SYMPTOMS, LOW VITALITY AND SEXUAL DYSFUNCTION IN HYPOGONADAL MEN WITH THE METABOLIC SYNDROME: THE MOSCOW STUDY**

Farid Saad, DVM, PhD¹, Svetlana Kalinchenko, MD, PhD³, Yuliya Tishova, MD, PhD³, George Mskhalaya, MD³, Louis Gooren, MD, PhD² and Erik Giltay, MD, PhD⁵

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Poster #94

**EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON MARKERS OF THE METABOLIC SYNDROME AND INFLAMMATION IN HYPOGONADAL MEN WITH THE METABOLIC SYNDROME: THE PLACEBO-CONTROLLED MOSCOW STUDY**

Farid Saad, DVM, PhD¹, Yuliya Tishova, MD, PhD³, George Mskhalaya, MD³, Louis Gooren, MD, PhD² and Svetlana Kalinchenko, MD, PhD³

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(Presented By: Farid Saad, DVM, PhD)
GENISTEIN EXERTS INHIBITION OF HUMAN AND RAT TESTICULAR 3\'-HYDROXYSTEROID DEHYDROGENASE ACTIVITY
Hongyu Zhou, MD, Guo-Xin Hu, MD, Binghai Zhao, MD, Yanhui Chu, PhD, Benson Akingbemi, PhD, Zhi-Qiang Zheng, MD and Ren-Shan Ge, MD
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(Presented By: Hongyu Zhou, MD)

AN ASSESSMENT OF THE EFFICACY AND SAFETY OF INTRAMUSCULAR INJECTIONS OF 750 MG TESTOSTERONE UNDECANOATE (TU) IN HYPOGONADAL MEN WITH NO HISTORY OF PRIOR TESTOSTERONE REPLACEMENT THERAPY DURING A 34 WEEK TREATMENT PERIOD
Abe Morgentaler, MD, FACS, Christina Wang, MD and Mark Harnett, MS
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(Presented By: Abe Morgentaler, MD, FACS)

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Diego Florentin, MD, Maurita Carrejo, MS and Robert Tan, MD, MBA
Michael E. DeBakey VA Medical Center
(Presented By: Diego Florentin, MD)

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George Toth, MD
TGH
(Presented By: George Toth MD)

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Daniel B. Martinez-Arguelles, MD, Martine Guity, PhD, Barry Zirkin, PhD and Vassilios Papadopoulos, DPharm, PhD
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(Presented By: Daniel B. Martinez-Arguelles, MD)

THE IMPLICATION OF STEM CELL REGULATION IN MALE INFERTILITY FOLLOWING MULTI-CYCLE CISPLATIN TREATMENT
James Harman, Jessica Cobarrubia and John H. Richburg
(Presented By: James Harman)

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Pei-Li Yao, Yi-Chen Lin and John Richburg
(Presented By: Pei-Li Yao)

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Binghai Zhao, PhD, Guoxin Hu, professor, Yanhui Chu, professor, Zhiqiang Zhang, professor, Barry R. Zirkin, professor and Ren-Shan Ge, professor
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(Presented By: Binghai Zhao, PhD)

ESTROGEN INHIBITS SPERMATOGENESIS IN PUBERTAL MICE BY BLOCKING TESTICULAR DESCENT AND SUPPRESSING TESTOSTERONE
Gunapala Shetty, PhD, Connie Weng, MD; PhD and Marvin Meistrich, PhD
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(Presented By: Gunapala Shetty, PhD)
**Poster #105**

**EFFECTS OF METHOXYCHLOR AND 2,2-BIS(P-HYDROXYPHENYL)-1,1,1-TRICHLOROETHANE ON 3β-HYDROXYSTEROID DEHYDROGENASE AND 17β-HYDROXYSTEROID DEHYDROGENASE 3 ACTIVITIES IN HUMAN AND RAT TESTES**

Xiao-Heng Li, MS, Binghai Zhao, MD, Yanhui Chu, PhD, Benson T. Akingebru, MD, Zhi-Qiang Zheng, MD, Ren-Shan Ge, MD and Guoxin Hu, MD

(Presented By: Xiao-Heng Li, MS)

**Poster #106**

**SHORT-TERM CIGARETTE SMOKE EXPOSURE CAUSES OXIDATIVE STRESS IN LEYDIG CELL AND INCREASES GERM CELL APOPTOSIS**

Riyad Ellati, MD and Jeffrey Lysiak, PhD

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(Presented By: Riyad Ellati, MD)

**Poster #107**

**GENOME-WIDE APPROACHES TO IDENTIFYING SPERM BIOMARKERS OF TOXICANT EXPOSURE**

Sara Pacheco, BS, Christoph Schorl, PhD, E. Andres Houseman, PhD, Karl Kelsey, MOH, MD, Mark Sigman, MD and Kim Boekelheide, MD, PhD

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(Presented By: Sara Pacheco, BS)

**Poster #108**

**ENVIRONMENTAL ORGANOCHLORINES AND SEX CHROMOSOME DISOMY IN HUMAN SPERM**

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(Presented By: Melissa J. Perry, ScD, MHS)

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**INFERTILITY / ART / MALE CONTRACEPTION**

**Poster #109**

**REVERSIBLE SUPPRESSION OF SPERMATOGENESIS BY THE BIS-DICHLOORACETYLDIAMINE WIN 18,446 IS MEDIATED BY INHIBITION OF RETINOIC ACID ACTIVITY WITHIN THE TESTES**

John Amory, MD, MPH, Charles Muller, PhD, David Amory, MD, PhD, Michael Griswold, PhD, Chris Small, MS, Alex Goldstein, PhD and William Brunnen, PhD

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(Presented By: John Amory, MD, MPH)

**Poster #110**

**THE BENEFIT OF A LONG TESTICULAR VAS REMNANT IN A POST VASECTOMY PATIENT SEEKING A REVERSAL**

Shulpa Lamba, MD and Joel L. Marmar, MD

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(Presented By: Shulpa Lamba, MD)

**Poster #111**

**GHRELIN PREVENTS CISPLATIN-INDUCED SPERM DNA DAMAGE**

Jose Garcia, MD, Shannon Whirlinge, PhD, Victor Papuska, BS, Tripli Haider, BS, Roy Smith, PhD and Dolores Lamb, PhD

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(Presented By: Jose Garcia, MD)

**Poster #112**

**AN EVALUATION OF MALE FACTOR AS A CONTRIBUTING FACTOR TO UNEXPLAINED INFERTILITY**

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*UMDNJ, Robert Wood Johnson Med. School at Camden

(Presented By: Britney Katsoff, BA)

**Poster #113**

**OOCYTE QUALITY INFLUENCES THE EFFECT OF SPERM DNA FRAGMENTATION ON PREGNANCY CHANCES, AS DEMONSTRATED BY LOGISTIC REGRESSION ANALYSIS**

Rebeca Santiso, PhD, Nicolas Garrido, PhD, Jose L. Fernandez, PhD, Sandra Garcia-Herrero, PhD, Thamara Villoria, PhD and Marcos Meseguer, PhD

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(Presented By: Marcos Meseguer, PhD)

**Poster #114**

**WHY MEN DO NOT USE THEIR CRYOPRESERVED SEMEN**

Marique Sorel, Eline Zijlregtop and M.T.W.T Lock

(Presented By: Marique Sorel)

**Poster #115**

**DOES MORPHOLOGY IN SEMEN PARAMETERS INFLUENCE REPRODUCTIVE OUTCOMES IN PATIENTS UNDERGOING IUI CYCLES?**

Fernando Quintana, BScEd, Iratxe Penalba, BScEd, Zaloa Larreategui, BScEd, Fernando Ayerdi, BScEd, Guillermo Quea, MD and Jose Serna, MD, PhD

(Presented By: Fernando Quintana, BScEd)
Poster #116  PERCUTANEOUS EMBOLIZATION VARICOCELES NUMBER ONE TREATMENT? (10 YEARS OF FOLLOW-UP)
Marique Sorel, A.A.G.M. Giesbers and P.L.M. Vijverberg
(Presented By: Marique Sorel)

Poster #117  BIOLOGICAL PROCESSES (BP) STATISTICALLY AFFECTED (SA) IN FRESH SPERM SAMPLES (SS) FROM FERTILE DONORS (D) VS. INFERTILE MALE PATIENTS (IP) WITH A REPRODUCTIVE ROLE (RR) RELATED
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¹IVI Foundation; ²IVI Valencia; ³IVI Alicante; ⁴Genomix
(Presented By: Nicolas Garrido, PhD)
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ROLE OF GHRELIN AND LEPTIN IN MALE REPRODUCTION

Carolina Jorgez, PhD1, Shannon Whirledge, PhD2, Aysegul Sahin, BS1, Roy Smith, PhD2 and Dolores Lamb, PhD2

1 BCM Scott Department of Urology; 2 BCM Molecular and Cellular Biology Department; 2 BCM Huffington Center on Aging

Introduction and Objectives: Male fertility requires the integration of the hypothalamic-pituitary-gonadal axis pathways and networks regulating body homeostasis for proper testicular function. In addition to the gonadotropins and sex steroid hormones, the hormones regulating energy balance and metabolism, ghrelin (Ghs) and its antagonistic partner, leptin are implicated and sex steroid hormones, the hormones regulating energy balance and metabolism, ghrelin (Ghs) and, its antagonistic partner, leptin are implicated.

Methods: Wild type; single mutant ghs/-/-, ghsr/-/-, ob/ob; and double mutants ghs/-/-;ob/ob and ghsr-/-;ob/ob mice were examined. Testicular architecture in ghs/-/- and ghsr/-/- mice appears normal. In contrast, ob/ob mice are cryptorchid with disrupted testicular morphology, reduced spermatogenesis, and atrophied interstitial Leydig cells. Testicular histology of the ghs/-/-;ob/ob revealed seminiferous tubules with abundant mature sperm. The germ and somatic cells of these testis display normal cell associations and morphology. This suggests rescue of the spermatogenic phenotype, despite low testis weight and continued display of cryptorchidism and obesity. Surprisingly, spermatogenesis is complete in the presence of cryptorchidism in these double mutant mice. To evaluate their fertility, leptin-deficient or double mutant mice were mated with super-ovulated and regular females. Results: These crosses did not result in blastocyst production or litters within 6-months. However, when a 60-day release testosterone pellet was implanted in males, a litter was obtained in 1/3 of ob/ob and ghs/-/-;ob/ob mice. In contrast, HCG injections 3-days/week for 3 months did not improve fertility in these males. Thus, infertility may be a direct consequence of lower circulating levels of testosterone; which may also be responsible for cryptorchidism; since one of the ghs/-/-;ob/ob mice displayed evidence of almost complete testicular descent. Gene expression assays are in progress.

Conclusion: Current results provide new evidence for the coupling of metabolic signals to control spermatogenesis in the mouse. Improved understanding of these interactions has important implications in reproductive biology.

SMAD3 DOSAGE INFLUENCES TESTICULAR MATURATION

Catherine Itman, PhD1, Chin Wong, BSc, (Hons)2, David A. Jans, PhD1, Matthias Ernst, PhD2 and Kate A. Loveland, PhD1

1 Department of Biochemistry & Molecular Biology and Department of Anatomy & Developmental Biology, Monash University, Victoria, Australia; 2 Department of Biochemistry & Molecular Biology, Monash University, Victoria, Australia; 3 Ludwig Institute of Cancer Research, Melbourne, Victoria, Australia

Introduction: Establishment and maturation of the Sertoli cell population underpins adult fertility. At puberty, Sertoli cells transition from an immature, mitotic state to become post-mitotic, terminally differentiated cells; Sertoli cell number is thus fixed from puberty. As the capacity for sperm production is determined by the number of Sertoli cells present (Orth 1988), adult sperm output is defined by the extent of Sertoli cell proliferation before puberty. Activin A promotes the proliferation of immature Sertoli cells (Boitani 1995), whereas in post-mitotic cells, activin increases levels of maturation markers Gja1 and Serpina5 (Itman 2009). Activin can signal via Smad2 and Smad3. As immature Sertoli cells utilize Smad3 in response to activin but post-mitotic cells signal utilize Smad2 and Smad3 (Itman 2009), we hypothesized that Smad3 is important in prepubertal tests development.

Objective: To compare testsis growth and somatic and germ cell maturation in Smad3+/-, Smad3+/- and Smad3-/- mice.

Methods: Testes and serum were collected at 7 days post partum (dpp), 16 dpp and adulthood (10-13 weeks). Sertoli cell proliferation and maturation, Leydig cell development and germ cell differentiation were examined by histology, immunohistochemistry and quantitative PCR. Serum follicle stimulating hormone (FSH) was measured by radioimmunoassay.

Results: Testsis weight was reduced in juvenile Smad3+/- and Smad3-/- mice, indicating that a threshold Smad3 level is required for normal testsis growth. Serum FSH levels were not different to wildtype. Histological and molecular analyses identified advanced Sertoli cell development in Smad3+/- and Smad3-/- mice but delayed maturation in Smad3+/- mice. Consistent with these data, germ cell maturation was advanced in Smad3+/- testsis, with round spermatids present at 16 dpp. Leydig cell development appeared unaffected by genotype. Adult Smad3+/- and Smad3-/- mice were fertile, but had smaller testes.

Conclusion: This is the first study relating Smad3 levels to puberty and identifies the Smad3+/- mouse as a model of peripheral precocious puberty. Thus, Smad3 must be appropriately regulated for the normal proliferation and timely maturation of Sertoli cells. Factors influencing Smad3 production will therefore affect testis maturation. We propose that the impact of environmental toxicants and endocrine disruptors which alter pubertal timing should be examined for their influence on Smad3 expression and related signaling events.

ELMO1 FUNCTIONS IN SERTOLI CELL-MEDIATED APOPTOTIC GERM CELL PHAGOCYTOSIS AND HOMEOSTASIS IN THE TESTES

Michael Elliott, PhD, Daeho Park, PhD, Robin Woodson, Shuju Zheng, PhD, Michael Reardon, Kodi Ravichandran, PhD and Jeffrey Lysiak, PhD

University of Virginia

Introduction: The efficient phagocytic clearance of apoptotic germ cells is the final step in the apoptosis program and is crucial for mammalian development and homeostasis, particularly in tissues with high rates of cell death. In the murine testis it is estimated that up to 75 percent of potential spermatogonia die via apoptosis; however, very few apoptotic germ cells are observed. Sertoli cells are the key phagocyte responsible for the engulfment of apoptotic germ cells, but the signaling pathways that regulate this clearance are poorly understood. Here we examined the expression level and functional contribution of a key engulfment signaling pathway in Sertoli cells, Baf1i/ELMO1/Doc1, and its role in the clearance of apoptotic germ cells in vitro and in vivo.

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Methods: BA1 is phosphatidylserine receptor that recognizes apoptotic cells and interacts with ELMO via its cytoplasmic tail. ELMO and Dock form an intracellular complex that promotes the activation of the cytoskeleton regulator Rac during corpse internalization. We found that the components of this pathway are expressed in primary and transformed (TM4 and 15 P-) cells and interacts with ELMO via its cytoplasmic tail. ELMO and Dock form complexes with ADAM2 and ADAM3 in the testis, and ADAM6 is a sperm protein that was eluted off the anti-sulfotyrosine column. ADAM6 is a major contributor to the phenotype of Tpst2-null sperm. On the other hand, the ADAM family member ADAM6 was identified by mass spectrometry as a sperm protein that participates in sperm-egg membrane interactions by modified by TPST2, and changes in these proteins in Tpst2-null sperm may alter the ability to fuse with the egg plasma membrane. The decreased fusion ability of Tpst2-null sperm may be a result of a decrease in the sulfation of ADAM6, which is necessary for sperm-egg fusion.

Results: In mice with elmo1 specifically deleted in the Sertoli cells, via use of the Amh-cre mouse strain, we again found increased frequency of uncleared apoptotic germ cell numbers. These data demonstrate that Sertoli cells contain and utilize the evolutionarily conserved ELMO engulfment module and for the first time describe BA1 as a receptor on Sertoli cells responsible for the recognition of apoptotic germ cells leading to engulfment. Understanding the molecular mechanisms of germ cell corpse clearance will provide a better understanding of the role of Sertoli cells in spermatogenesis.

Conclusion: Taken together, these data indicate that Izumo is unlikely to be a major contributor to the phenotype of Tpst2-null sperm. On the other hand, the ADAM family member ADAM6 was identified by mass spectrometry as a sperm protein that participates in sperm-egg membrane interactions by modified by TPST2, and changes in these proteins in Tpst2-null sperm may alter the ability to fuse with the egg plasma membrane. The decreased fusion ability of Tpst2-null sperm may be a result of a decrease in the sulfation of ADAM6, which is necessary for sperm-egg fusion.

Funding: Supported by NIH-NICHD HD 052035.
ROLE OF ENKURIN IN MALE FERTILITY
Melissa Jungnickel, PhD, Keith Sutton, PhD and Harvey Florman, PhD
LMass Medical School, Dept Cell Biology
(Presented By: Melissa Jungnickel, PhD)

Introduction and Objectives: Ca2+ signaling in sperm plays a key role in the regulation of events preceding fertilization. In ejaculated spermatozoa, family member, TRPC2, is involved in the ZP3-driven acrosome reaction of both mouse and human sperm. We have previously found that one testis and vomeronasal organ, and in mouse sperm, is localized to both the intracellular Ca2+ concentration ([Ca2+]i) is a key player in the process type controls. Examination of live sperm under light microscopy reveals a sperm acrosomal crescent and flagellum, suggesting a potential role in capacitation, regulating motility, hyperactivation, chemotaxis, and the acrosome reaction. The transient receptor potential canonical (TRPC) family of cation channels mediate a wide range of Ca2+-dependent processes. There are seven mammalian TRPCs, several of which, are expressed in the head and tail of both mouse and human sperm. We have previously found that one family member, TRPC2, is involved in the ZP3-driven acrosome reaction in mouse sperm. In order to identify TRPC2-interacting proteins that might be candidate regulators or effectors in downstream signaling events, we carried out a yeast two-hybrid screen. This strategy led to the identification of enkurin, a novel. Multi-domain protein containing a C-terminal domain that binds several TRPCs, and a proline-rich N-terminal region with predicted SH3 ligand motifs. Enkurin also contains an IQ motif that binds Ca2+-calmodulin, and therefore associates a Ca2+-sensor with the inner face of a Ca2+-conducting ion channel. Enkurin is expressed at high levels in the testis and vomeronasal organ, and in mouse sperm, is localized to both the sperm acrosomal crescent and flagellum, suggesting a potential role in capacitation, the acrosome reaction and sperm motility.

Methods: To examine the effect of enkurin removal on sperm function, we have generated a gene deletion mouse model.

Results: Initial phenotypic studies indicate that while female nulls show normal reproductive function, male mice homozygous for mutations in the enkurin allele have poor sperm motility and severely compromised fertility; enkurin null males show a 96% reduction in litter size relative to the wild-type controls. Examination of live sperms under light microscopy reveals a significant difference between the mutant and wild-type sperm. Sperm from wild-type mice display vigorous tail beating and fast progressive directed movement. Conclusion: By contrast, enkurin +/- sperm are either sluggish or immotile and displayed less directed motion. We are currently working to identify the basis of the defect in enkurin null males.

Sunday, April 11, 2010
2:00 p.m. – 3:30 p.m.

Oral Session II - Clinical Location: Regency Ballroom C Moderators: John K. Amory, MD • Shayne Louis, MD

RELIABILITY OF ANOGENITAL DISTANCE MEASUREMENT AS AN ASSAY OF MALE FERTILITY
Akanksha Mehta, MD1, Kim Boekelheide, PhD2 and Mark Sigman, MD3
1Division of Urology, Warren-Alpert School of Medicine at Brown University; 2Warren-Alpert School of Medicine at Brown University
(Presented By: Akanksha Mehta, MD)

Introduction and Objectives: Anogenital distance (AGD) is sexually dimorphic in many mammals, with males having a longer AGD on average than females. It has been suggested that prenatal exposure to chemicals with anti-androgenic properties can decrease AGD, and may lead an increased incidence of infertility, hypospadias, cryptorchidism, and testicular cancer in human males. AGD in infants has been demonstrated to be a reliable measurement and has been found to be inversely correlated with phthalate concentrations in maternal urine. We designed a prospective study to determine the reliability of AGD measurements in adult males and to compare AGD between groups of fertile and infertile men.

Methods: Patients presenting for urologic evaluation were divided into two groups after informed consent. Group A consisted of fertile men presenting for elective vasectomy who had achieved paternity within the preceding 2 years. Group B consisted of men presenting for evaluation for infertility. All patients underwent a complete history and physical exam, and laboratory testing as indicated. AGD was measured by summing the distance from mid anus to the junction of the scrotum and perineum (ano-scrtal distance, AS) and the distance from the junction of the scrotum and perineum and the penoscrotal junction (peno-scrtal distance, PS). To determine measurement reliability, the fertile patients underwent repeat AGD measurements at a second office visit by the same physician.
Results: Group A consisted on 25 fertile men, while group B consisted of 66 men. The measurement of AGD was not reliable with the intra- patient variation being as large as the interpatient variation (intraclass correlation coefficient = 0.55). AGD indices calculated by dividing AGD by height, weight, or body mass index did not improve the reliability of the AGD measurement. In addition, neither AS, nor PS measurements performed better than AGD. No significant difference was noted in the mean AGD values between fertile and infertile men (136.3 vs. 139.4, p=0.438). The ratios of AGD/BMI and AGD/height also demonstrated no significant difference between fertile and infertile men (4.96 vs. 4.87, p=0.993; and 1.95 vs. 2.01, p=0.369 respectively).

Conclusion: Unlike in infants, AGD is not a reliable or reproducible measure in adult males. No significant difference in AGD measurements was seen between fertile and infertile patients.

GENETIC AND HORMONAL CONTROL OF BONE VOLUME,
ARCHITECTURE AND HISTOMORPHOMETRY IN XXY MICE
Peter Liu, MBBS, (Hons) PhD1, Robert Kalak, PhD2, YanHe Lue, PhD3, Krista Erkkila, MD, PhD4, Yue Jia MD3, Hong Zhou, PhD3, Markus Seibel, MD3, Christina Wang, MD1, Ronald Swerdloff, MD3 and Colin Dunstan, PhD2
*Woolcock Institute of Medical Research, University of Sydney; 2University of Sydney; 3Harbor-UCLA Medical Center; 4Hospital for Children and Adolescents, University of Helsinki

Introduction and Objectives: Klinefelter’s syndrome is the most common chromosomal aneuploidy in men (XXY karyotype, 1:600 live births) and results in testicular (infertility and androgen deficiency) and non-testicular (cognitive impairment and osteoporosis) deficits. The extent to which skeletal changes are due to testosterone deficiency or arise directly from gene over-dosage cannot be easily determined in humans. To answer this, we generated XXY mice through a 4-generation breeding scheme.

Methods: Eight intact XY and 9 XXY littermate controls, and 8 castrated XXY mice and 8 castrated XY littermate controls, were sacrificed at one year of age. Castration occurred 6 months prior to sacrifice. A third group of 9 XXY and 11 XY littermates were simultaneously castrated and implanted with a 1 cm silastic testosterone capsule 8 weeks prior to sacrifice. Tibiae were harvested from all three groups and examined by microCT and histomorphometry. Blood testosterone was assayed by radioimmunoassay.

Results: Compared to intact XY controls, XXY mice had lower bone volume (6.8±1.2 vs 8.8±1.7%, mean±SD, P=0.01) and thinner trabeculae (50±4 vs. 57±5um, P=0.007). Trabecular separation (270±20 vs. 270±20um), osteoclast number relative to bone surface (2.4±1.0 vs 2.7±1.51 mm2) and blood testosterone concentrations (5.3±4.7 vs 2.5±3.9 ng/mL) did not differ significantly. Testosterone replaced XXY mice continued to show lower bone volume (5.3±2.4 vs 8.1±3.5%, P=0.026), greater trabecular separation (380±69 vs. 324±62um, P=0.040), but equivalent blood testosterone (6.8±1.2 vs 8.8±1.7%, mean±SD, P=0.01) and trabecular width (P<0.001) to such a great extent that differences between XXY and XY mice were undetectable.

Conclusion: In conclusion, XXY mice replicate many features of human Klinefelter’s syndrome and are therefore a useful model for studying bone, as well as testis and behavior. Testosterone deficiency does not explain the bone phenotype because testosterone replaced XXY mice show reduced bone volume despite similar blood testosterone levels. These data suggest that novel genes, that escape X inactivation, contribute to bone loss, and when identified, may provide unique molecular targets for the management of osteoporosis.


ENHANCEMENT OF VITAMIN D BASED THERAPY FOR PROSTATE CANCER TREATMENT BY SELECTIVE TARGETING OF 24-HYDROXYLASE
Mounia Tannour-Louet, PhD, Shaye Lewis, PhD, Julie Stewart, PhD, Aysegul Sahin, BS, Shuo Han, BS, Josephine Addai, BS and Dolores Lamb, PhD
Baylor College of Medicine

Introduction: The hormonal form of vitamin D3, 1,25-dihydroxyvitamin D3, 1,25(OH)2D3, exerts anti-proliferative, anti-angiogenic and pro-apoptotic effects on prostate cancer cells. Clinical trials are under evaluation to develop vitamin D3-based therapies for prostate cancer treatment. However, upper level dose limits are critical since effective therapeutic levels may produce hypercalcemia, which can result in coma and cardiac arrest.

Objectives and Methods: To obtain optimal cancer cell growth abrogation without affecting the blood calcium levels, we propose to achieve a higher intratumoral exposure of vitamin D3 by selectively inhibiting the tissular expression of the main catabolic enzyme of vitamin D3, Cyp24, using RNA interference.

Results: Abrogation of Cyp24 expression using specific siRNA in human prostate cancer cell lines (LnCAP, DU145 and PC3) significantly enhanced the 1,25(OH)2D3-mediated growth inhibition as assessed by proliferation assays and the expression profile of key markers of cell proliferation including p21, p27 and cyclinD1. LnCAP, DU145 and PC3 stable cell lines expressing Cyp24 shRNA or non-targeting shRNA were generated and used in xenograft-bearing mice subjected to low doses of vitamin D3 supplementation. Assessment of the in vivo efficiency of the anti-proliferative action of vitamin D3 in presence of selective inhibition of Cyp24 is ongoing. In addition, we found that Cyp24 protein was overexpressed in human adenocarcinomas of prostate and that the relative mRNA levels of Cyp24 increased with increasing pathological grades of prostate cancer.

Conclusion: These findings suggest that increased Cyp24 expression in human prostate cancer tissues presumably decreases the intratumoral 1,25(OH)2D3 levels, effectively counteracting the anti-proliferative effects of vitamin D3 by selectively inhibiting Cyp24 expression, vitamin D action may be enhanced and therapeutic doses could then be lowered in the treatment and chemoprevention of prostate cancer.

RHOA/RHO-KINASE-MEDIATED CA2+ SENSITIZATION AND CA2+ ENTRY VIA L-TYPE VOLTAGE-GATED CA2+ CHANNELS MAINTAIN HUMAN CANVEROSMAL TISSUE ON ACTING DIRECTLY ON NORADRENERGIC NEURONS IN THE FLACCID STATE
Suresh Gur, PhD, Philipp Kadowitz, PhD, Suresh Sikka, PhD and Wayne Hellstrom, MD, FACS

Introduction and Objectives: Penile corpus cavernosum maintains its contractile (flaccid) state by release of noradrenaline from noradrenergic nerves. Nevertheless, it is uncertain which intracellular pathways mediate these neurogenic contractile effects in the human corpus cavernosum (HCC). We investigated the underlying mechanisms of adrenergic-mediated contractile responses in HCC.

Methods: HCC specimens were obtained from patients undergoing penile prosthesis surgery (age range 60–71 yrs., n = 6) with local institutional approval. Strips (four from each HCC, 1 x 1 x 6 mm) were suspended in an organ bath containing Krebs-Henseleit solution at 37 degrees C and pH 7.4, constantly bubbled with 95% oxygen and 5% CO2. Electric field stimulation (EFS, 0–80 Hz, train rate 10 ms, 1ms duration, 1ms delay, 150V) of strips at baseline level was delivered via platinum wire electrodes. Frequency-dependent contractile responses were observed after preincubation with atropine (10 µM) plus L-NAME (100 µM) to eliminate cholinergic and nitricergic responses. Frequency-response curves to EFS were generated in the absence and presence of Rho-kinesin (ROCK) inhibitor, fasudil [HA1077, 1-(5-isooquinolinesulfonyl)-homopiperazine, 1 µM], Ca(2+) channel antagonist (isradipine, 3 µM) or nonspecific α-adrenergic receptor (AR) blocker phentolamine (10 µM) or sodium nitrite (NaNO2, 1 mM).

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that recapitulate the expression profile of endogenous markers of male
Anomalies were scattered across the genome but notably clustered in gene­
enriched subtelomeric loci.

Increased the gene expression of Hoxa13 and its downstream target Fgf8,
Vamp7 gene expression was not affected, its protein levels increased in
the effect of testosterone on the expression of Vamp7 in NTERA-2 cells
S!X

ambiguous genitalia and 10 p14 and Xq28 for cryptorchidism and 12 p13,
as these GU defects may result from defective androgen signaling. While

Conclusion: Taken together, the identification of these clinically significant copy number variants will help to elucidate the molecular mechanisms underlying the pathogenesis of human genital development and define critical factors such as VAMP7 in male sexual development.

Results: The maximum response (0.72 ± 0.16g, at 80Hz) to EFS showed
65% decrease by fasudil and 70% decrease by isradipine (Figure). The α-AR antagonist phenolamine nearly abolished (98%) by EFS-induced contractions compared to 21% decrease following preincubation with NaNO2.
Conclusion: Neurogenic (sympathetic) contractions are not only caused by
an increase of Ca2+ influx via L-type voltage-gated Ca2+ channels alone, but also by an increase in Ca2(2+) sensitivity via ROCK pathway. The ROCK inhibitor fasudil and Ca2+ channel blocker isradipine had similar effects suggesting parallel mechanisms in HCC that do not involve the nitrergic system. These other pathways may serve ED patients with little nitrergic function and who do not respond to phosphodiesterase inhibitors.

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IDENTIFICATION OF COPY NUMBER VARIANTS ASSOCIATED TO GENITOURINARY BIRTH DEFECTS AND CHARACTERIZATION OF NOVEL KEY GENES IN HUMAN GENITAL DEVELOPMENT
Shuo Han, Mounia Tannour-Louet, PhD, Sean Corbett, MD and Dolores Lamb, PhD
Department of Urology, Baylor College of Medicine
(Presented By: Shuo Han)

Introduction and Objectives: Congenital genitourinary abnormalities such as hypospadias, cryptorchidism and ambiguous genitalia are among the most common human birth defects. However, the etiology of these reproductive abnormalities remains poorly understood and, therefore, highlights the need to define the molecular protagonists and regulatory pathways governing genitourinary (GU) development. Submicroscopic chromosomal anomalies have been attributed as the molecular basis of genomic syndromes such as mental retardation, developmental delays and heart defects. We hypothesize that abnormal urogenital tract development result from similar chromosomal aberrations that cannot be detected by a routine karyotype.

Methods: A clinically validated comparative genomic hybridization microarray was used to identify the existence of significant copy number variants that completely evaded detection by high resolution karyotype in children born with cryptorchidism, hypospadias or ambiguous genitalia. Anomalies were scattered across the genome but notably clustered in gene-enriched subtelomeric loci.

Results: Confirmed de novo duplication and deletion events were significantly associated with GU defects (P=1.02x10^-26) as compared to 15,931 non-GU patient controls on 1p36.33, 9p23p24 and 19q12-q13.11 for ambiguous genitalia and 10p14 and Xq28 for cryptorchidism and 12p13, 16p11.2 and 16q24.3 for hypospadias. Of note, a gain on Xq28 was found at the plasma membrane of halo cells. Multiple transcripts for Panx1 were identified and sequence analysis indicates that alternative splicing may account for these isoforms. One of these isoforms lacks exon 3, which codes for one of the transmembrane domains of the protein. Whether or not the resulting protein is functional remains to be established.

Results: Panxs are glycoproteins and their degree of glycosylation is thought to be important for their cellular localization. The extent of glycosylation of both Panxs in the epididymis appears to be regulated by testicular androgens, which repress glycosylation. This is the first observation that Panx glycosylation can be regulated.

Conclusion: In conclusion, Panxs are expressed in the epididymis and are regulated by both alternative splicing and via androgen-dependent glycosylation. Considering the role of pannexins in A TP secretion, these proteins may play a significant role in ATP secretion into the epididymal lumen by principal cells.

Funding: Supported by NSERC and CIHR.

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REGULATION OF PANNEXIN-1 AND PANNEXIN-3 IN THE ADULT RAT EPIDIDYMIS
Daniel Cyr, PhD¹, Patrick Turmel, BSc², Julie Dufresne, MSc³, Charles Smith, PhD³, Silvia Penuela, PhD³, Dale Laird, PhD³, and Louis Hermo PhD³
¹INRS-Institut Armand-Frappier; ²McGill University; ³University of Montreal; ⁴University of Western Ontario
(Presented By: Daniel Cyr, PhD)

Introduction and Objectives: Pannexins (Panxs) are recently identified members of the gap junction family of proteins. Although homologous to invertebrate gap junction proteins, the innexins, they appear to form hemichannels, or pannexons, that are implicated in ATP secretion. Three members of the Panx family have been identified.

Methods: To evaluate the regulation of Panxs in the male reproductive tract, we investigated the expression and distribution of Panx-1 and -3 in the testis, efferent ducts, and epididymis of adult rat. In the testis, Panx1 was localized to the basal compartment of the seminiferous epithelium, and Panx3 was localized to Leydig cells. In efferent ducts, both Panxs were expressed in the apical region of ciliated cells while in the epididymis, both Panxs were expressed in principal cells and prominent labelling for Panx1 was observed between principal and basal cells. Panx3 was also found at the plasma membrane of halo cells. Multiple transcripts for Panx1 were identified and sequence analysis indicates that alternative splicing may account for these isoforms. One of these isoforms lacks exon 3, which codes for one of the transmembrane domains of the protein. Whether or not the resulting protein is functional remains to be established.

Results: Panxs are glycoproteins and their degree of glycosylation is thought to be important for their cellular localization. The extent of glycosylation of both Panxs in the epididymis appears to be regulated by testicular androgens, which repress glycosylation. This is the first observation that Panx glycosylation can be regulated.

Conclusion: In conclusion, Panxs are expressed in the epididymis and are regulated by both alternative splicing and via androgen-dependent glycosylation. Considering the role of pannexons in ATP secretion, these proteins may play a significant role in ATP secretion into the epididymal lumen by principal cells.

Funding: Supported by NSERC and CIHR.

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INFLUENCE OF MERCURIC CHLORIDE ON ANTIOXIDANT SYSTEM IN THE TESTIS AND EPIDIDYMIS OF ALBINO RATS
Venugopal Ramalingam, MSc, MPhil, PhD¹, Suganthi Onserine Marcelline, MSc, MPhíl, ME², Subbarayalu Panneerdoss, MSc, MPhil, PhD³ and Viswanadhappallli Suryavathi, MSc, MPhil, PhD³
¹Lecturer; ²Scientist
(Presented By: Venugopal Ramalingam, MSc, MPhil, PhD)

Introduction and Objectives: Environmental exposure to heavy metals is associated with a wide range of toxic effects. It is evident that heavy
metals released in the environment affect the reproductive processes and fertility of animals. Mercury is used in agriculture as fungicide, in medicine as topical antiseptic and disinfectant and in chemistry as an intermediate in the production of other mercury compounds. Toxic metals affect the male fertility of animals. Mercury is used in agriculture as fungicide, in medicine as topical antiseptic and disinfectant and in chemistry as an intermediate in the epididymis was removed. Antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase were estimated in the epididymis extract. Lipid peroxidation, hydrogen peroxide, vitamin C and vitamin E were also estimated. Mercuric chloride administration had no effect on the body weight of the animals but the weight of the testis and epididymis was decreased.

Conclusion: The present study suggests the reproductive toxicity of mercury by inducing the oxidative stress in the testis and epididymis and possible interference in sperm production and further maturational processes.

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TESTICULAR LUMINAL FLUID FACTORS REGULATE MAPK, JAK/STAT AND NFkB PATHWAYS IN THE INITIAL SEGMENT OF RAT EPIDIDYMIS TO PREVENT CELL APOPTOSIS

Bingfang Xu, Rana Abdel-Fattah, Ling Yang, Sallie Crenshaw and Barry Hinton

(Presented By: Bingfang Xu)

Introduction and Objectives: The initial segment of the epididymis is vital for male fertility, therefore, it is important to understand the mechanisms that regulate this important region. Without testicular luminal fluid factors, e.g. FGFs, a subset of cells within the initial segment undergo apoptosis. This study was undertaken to examine early changes in the downstream signal transduction pathways following loss of testicular luminal fluid factors.

Methods: Initial segments were removed from rats that had undergone efferent duct ligation (EDL) and a combination of microarray analyses and Kinexus screens were used to reveal the following cascade of events leading to loss of protection and eventual apoptosis. In the first 6h of loss of testicular luminal fluid factors, down regulation of MAPK pathway components was observed at the mRNA and protein activity levels.

Results: Microarray analysis revealed that mRNA expression of Dusp6 and Dusp5—two key components of MAPK pathway—decreased 2.5-3.4 times, while kinexus screen revealed the activities of MEK and ERK declined. Immunohistochemistry staining of phospho-ERK indicated that down-regulation of the MAPK pathway was specific to the epithelial cells of the proximal initial segment. Subsequently, after loss of testicular luminal fluid factors for 12h, levels of mRNA expression of JAK/STAT and NFkB pathways components increased, mRNA expression of genes encoding cell cycle repressors increased and levels of protein expression of several pro-apoptotic phosphatases increased. Finally, after 18h of loss protection from testicular luminal fluid factors, apoptosis was observed.

Conclusion: In conclusion, testicular luminal fluid factors protect the cells of the initial segment by activating MAPK pathway, repressing JAK/STAT and NFkB pathways, and preventing a cascade of reactions leading to apoptosis.

Funding: Supported by NIH—NICHD HD 052035.

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PROTEOMIC ANALYSIS OF GUINEA PIG SPERM ACROSOMAL CONTENTS AND HYBRID VESICLE MEMBRANES

Vincent Arnone¹, Alex Johnson¹, Mariano Buffone, PhD², George Gerton, PhD³ and James Foster, PhD⁴

¹Randolph-Macon College; ²University of Pennsylvania

(Presented By: Vincent Arnone)

Introduction and Objectives: A functional sperm acrosome is necessary for fertilization in mammals, yet many questions remain about the function of this organelle and the proteins it contains. The guinea pig was used as a model in this study because the acrosome is very large, and well-characterized methods exist to obtain the acrosomal contents and membrane vesicles (i.e. "hybrid vesicles" that consist of plasma and outer acrosomal membranes).

Methods: We induced acrosomal exocytosis with a calcium ionophore and recovered the highly enriched acrosomal contents and hybrid vesicles by sequential centrifugation. Proteins were separated by one- and two-dimensional SDS-PAGE, and proteomic analysis of the excised bands and spots was performed to obtain peptide sequences by mass spectrometry.

Results: We found a dozen proteins known to be present in the acrosome (e.g. CRISP-2, ZP3R, AM50, proacrosin, granulins) and several dozen proteins that are not well-characterized or have not been previously described as acrosomal or sperm associated. When categorized by function, the proteins are mainly involved in egg-binding, hydrolase, regulatory functions, signaling and structure, although a major group (about 20%) contained proteins of unknown function. Also, acrosomal matrix proteins were detected in both the acrosomal contents and hybrid vesicle fractions; this confirms the finding in previous morphological studies that acrosomal matrix proteins appear to remain associated with the hybrid vesicles during acrosomal exocytosis. Currently several proteins are under further study by immunofluorescence microscopy and Western analysis to verify their presence in the acrosomal region.

Conclusion: In conclusion, this study provides a thorough analysis of the major acrosomal content and hybrid vesicle proteins, and further evaluation of the novel proteins will provide new insights into the function of the acrosome.

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**17**

THE DIFFERENT DISTRIBUTION OF SMALL RNAs OF SOMATIC AND GERM LINE CELLS IN HUMAN TESTES

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(Presented By: Eitetsu Koh PhD, MD)

Introduction and Objectives: Small RNAs are well characterized that they are highly involved in post-transcriptional regulation and control with developments and widely related with regulation role. Here we also try to explore their expression patterns between somatic and germ cells in human testes.

Methods: To define the expression of small RNAs, we isolated total RNAs from testes of Sertoli only syndrome (SCO) patient and normal spermatogenesis male (NS) confirmed by histological examination. Consequently, collected sequences spanned on 19-31nt from RNAs and read sequences using GS-FLX. Finally 19.093 (SCO) and 36,481 (NS) reads were analyzed for screening and genome blast (NCBI build up 2.2).
**FILLING THE HOLES IN THE HUMAN GENOME: DISCOVERY OF THE SPERM ACROSOMAL PROTEIN SSMP20**

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**Introduction and Objectives:** The molecular processes underlying sperm-egg interactions are not fully understood partly because of difficulties in comparing results obtained using diverse experimental methods and different animal models. Because sperm-specific membrane proteins (SSMPs) and lipid raft proteins (SSLRPs) are likely to have unique functions in gamete interactions, we tested whether new proteins with functions in fertilization could be identified by systematically characterizing sperm membrane alloantigens in a single species.

**Methods:** Female pigs with a high alloimmune response to sperm membranes (TWM) or lipid rafts (LR) gave birth to fewer offspring than non-immune control animals (81 and 66% decreases, respectively; \( p = 0.0015 \)), suggesting that at least one SSMP and SSLRP functions in fertilization. Alloantiseria to TWM or LR detected ~30 SSMPs and 5 unique SSLRPs on 2-D immunoblots.

**Results:** Major membrane alloantigens identified by mass spectrometry were the ADAMs fertilin alpha, fertilin beta, and cyritestin. Minor alloantigens included myo-inositol monophosphatase-1 and GP-2. Immunodominant SSLRPs included the epididymal sperm protein E12 and SAMP14. De novo peptide sequences of an Mr 20,000 alloantigen (SSMP20) matched no known sequences. A partial length (436 bp) 3'-end cDNA was amplified from pig testis by PCR with degenerate oligonucleotide primers, and the remainder was amplified with specific primers by 5'-RACE. Northern blots confirmed that the 1.1 kb composite cDNA was full length and testis-specific. The cDNA encoded a 126 amino acid protein with two predicted transmembrane segments and no signal peptide. BLAST searches with the deduced SSMP20 sequence identified a single mouse cDNA and a single hypothetical gene product from macaque. The corresponding genomic loci spanned Upk1a-Ffar2 of mouse Chr 7 and macaque Chr 19.

**Conclusion:** Sequences of bovine, horse, human, and rat orthologs were then identified as conserved elements not previously annotated in syntenic regions of the respective genomes. SSMP20 localized to the acrosome of fixed pig spermatozoa in immunofluorescence, but was not detected on live-labeled cells. Immunoelectron microscopy refined the location of SSMP20 to the inner leaflet of the plasma membrane, the outer acrosomal membrane, and in the acrosome. We conclude that SSMP20 is a potential fertilization protein in the sperm head that was not correctly identified in the genomes of five species, including human.
Methods: INSL3/RXFP2 or androgen receptor gene ablation causes cryptorchidism in transgenic mice. Mutations of orthologous genes were identified in human patients with testicular maldescent. The effect of RXFP2/INSL3 gene dosage appears to be species-specific as human patients but not mice heterozygous for mutant allele manifest the abnormal phenotype. To analyze the effects of hormonal signaling disruption we produced a novel mouse line with Cre-loxP activated shRNA transgene targeting RXFP2 gene expression (shRXFP2) and the mice with conditional knockout of androgen receptor in gubernacular ligaments (ARKO-GU). We have demonstrated that in shRXFP2 transgenic gubernacula the expression of RXFP2 gene is significantly down-regulated. The abnormal testis phenotype was observed only in shRXFP2,Rxfp2+/- males, suggesting the gene-dosage effect observed in human patients.

Results: Inactivation of androgen receptor in gubernacula caused similar cryptorchid phenotype, thus identifying target tissue for androgen signaling in testicular descent. The alterations of global gene expression in mutant vs wild type gubernacula was performed using Illumina oligonucleotide arrays. Despite that a number of genes were significantly changed in shRXFP2 mice the pattern of gene expression in two models was significantly different. While in shRXFP2 mutants Gene Ontology analysis revealed that 30% of misregulated genes were involved in developmental processes, the majority of abnormal gene expression in ARKO-GU mice was related to cell signaling. The expression of significant number of the genes of Wnt/beta-catenin and Notch signaling in RXFP2-deficient gubernacula was altered, suggesting the local regulation of these pathways by INSL3 in gubernacular cells.

Conclusion: In summary, our data confirmed that the gubernacular ligament is a primary target of hormonal signaling during testicular descent and identified potential downstream targets of INSL3 and androgen hormones in gubernacular cells.

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FUNCTIONAL ASSESSMENT OF CRYOPRESERVED BOVINE GONOCYTES: ISOLATED CELLS VS TISSUE

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(Presented By: Michael Holland, PhD)

Introduction and Objectives: Germ cell transplantation is an important experimental tool to study the stem cell niche in the testis. Since bovine gonocytes cannot be maintained for long term in cell culture they must be isolated from tissue. Unfortunately, bull calves of the right age are not available year round. Therefore we compared transplantation success of freshly isolated gonocytes with those isolated from frozen tissue.

Methods: Two testes, (3-5g), from calves, containing only gonocytes, were minced for cell isolation. The cells were frozen in liquid nitrogen. One testis was cut into 2-5 mm3 (5-40 mg) pieces which were either subcutaneously grafted in the back of nude mice or frozen to -196°C for between 24 hours to 5 months. The cryopreserved tissue was either grafted into nude mice or used to isolate gonocytes for transplantation into nude mouse testes. We compared the ability of the two populations of gonocytes to colonize and proliferate. Transplantation of bovine gonocytes isolated from fresh testes was the positive control. The percentage of tubules showing DBA (germ cells) or vimentin (Sertoli cells) positive cells were counted in 3-4 sections, 500μm apart, per testis. Based on the viability, density and volume of donor cells injected into each testis, the percentage of tubules having bovine germ cells or Sertoli cells in the mouse recipient testis per section and per 104 viable cells injected was calculated.

Results: In both fresh and frozen-thawed bovine tissues implanted subcutaneously the germ cells survived and differentiated. Transplanted gonocytes isolated from both fresh and frozen tissue migrated to the basement membrane from central lumen of the tubules. Results showed the number of colonizing loci of germ cells from the cryopreserved bovine testis tissues was higher (P <0.05) than that of the isolated cryopreserved cells (1.57 ± 0.46 % v 0.28 ± 0.12% tubules /104 viable cells). The efficiency of Sertoli cells surviving and colonizing in mouse tubules was similar for fresh and cryopreserved cells (0.34% v 0.36% tubules /104 viable cells).

Conclusion: We conclude the ability of bovine gonocytes collected from cryopreserved bovine testis tissues to colonize the mouse nude testis was superior to that of isolated cryopreserved gonocytes. The preservation of bovine testis tissue slices is a reliable way to preserve gonocytes for subsequent isolation, culture and transplantation. The validity in species other than the bovine remains to be tested.

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EXPRESSION OF C-KIT MRNA AND KIT PROTEIN DIFFERS IN SPERMATOGENIAL STEM CELLS BEFORE AND AFTER DIFFERENTIATION IN MOUSE

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(Presented By: Lei Zhang)

Introduction: KIT belongs to a family of growth factor receptors with intrinsic tyrosine kinase activity that transduces growth regulatory signals across the plasma membrane and is composed of an extracellular, a transmembrane and an intracellular domain. The 21-exon gene encodes a 5.5 kb full-length transcript. Eexpression of c-kit in the spermatogenic cells is periodic as they differentiate towards functional sperm. The spermatogenic cells are originated from spermatogonial stem cells (SSCs), which are also called type A spermatagonia. C-kit expression is re-initiated in type B (differentiated) spermatagonia. Several isoforms of c-kit transcript have been discovered, including a long isoform (exon 1–21), a truncated isoform (exon 13-21) and a soluble isoform (exon 1–9). The truncated isoform is discovered to be expressed only in the spermatids. However, the transcription and translation of c-kit in SSCs before and after differentiation is still unclear.

Objectives: To reveal and compare the c-kit expression profile in the mouse spermatogonial stem cells before and after commitment of differentiation.

Methods: A type A spermatogonial cell line (C18-4, representing the undifferentiated stem cells) and a type B spermatogonia cell line (CRL-2053, representing the differentiated stem cells) are studied simultaneously. Total RNA of these two kinds of cells are extracted and the transcription of c-kit is examined by RT-PCR using primers designed against regions spanning exon 2–4, exon 11–14 and exon 19–21 respectively. Rapid amplification of cDNA ends (RACE) is applied to analyze the 5' and 3' end of c-kit gene in the 2 kinds of cells.

Results: The full-length transcript and protein are not expressed in SSCs before differentiation and are expressed in those after differentiation. However, a shorter transcript (starting from exon 10) of c-kit is expressed in the SSCs before differentiation as revealed by RACE.

Conclusion: There are more than 2 isoforms of c-kit transcript existed in the SSCs. There are dynamic transcription changes of these isoforms before and after differentiation. These transcription changes might be either causes or consequences of differentiation. The study is now actively studied in our lab.

Funding: This study is supported by CUHK direct grant (2041472) and Hong Kong RGC grant (CUHK 464809) to Y Han.

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FERTILIZATION DEFECTS IN SPERM FROM TYROSYLPROTEIN SULFOTRANSFERASE 2 (TPST2)-DEFICIENT ANIMALS ARE LINKED WITH ABNORMALITIES IN A DISINTEGRIN AND A METALLOPROTEASE (ADAM) PROTEINS AND NOT ABNORMALITIES IN IZUMO

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(Presented By: Matthew Marcello)

Introduction and Objectives: Tyrosine O-sulfation is a posttranslational modification catalyzed by tyrosylprotein sulfotransferases (TPSTs) localized in the trans-Golgi network. Male mice deficient in TPST2 are infertile. Our past work showed that zona-pellucida (ZP)-free eggs inseminated with Tpst2-null sperm have fewer bound sperm but more fused sperm when compared to eggs inseminated with wild-type sperm (J. Biol. Chem. 281:9423). More recently, we have shown that: (1) the increased extent of sperm-egg fusion observed with Tpst2-null sperm is not due to a failure of these sperm to trigger the egg's establishment of a membrane block to polyspermy, (2) Tpst2-null sperm, once bound to the egg plasma membrane, have an increased ability to fuse with eggs as compared to wild-type sperm.
AGING CAUSES DIFFERENTIAL GENE EXPRESSION IN THE PACHYTENE SPERMATOCYTES IN THE BROWN NORWAY RAT
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Introduction and Objectives: There is an increasing number of parents who are deciding to have children at an older age. There is now evidence that the function of the male reproductive system declines with age. Fathers over the age of 50 have increasing problems with spermatogenesis that include decreased sperm motility and increased chromatin aberrations, leading to a decreased pregnancy rate in the partners of these older males. This study was undertaken to determine the effects of advancing age on changes in gene expression in pachytene spermatocytes, a key, highly sensitive phase of spermatogenesis.

Methods: Male Brown Norway (BN) rats represent a well-established model of male reproductive aging. Pachytene spermatocytes were recovered from rats at 4 months of age (young) and 21 months (aged) using the STA-PUT velocity sedimentation technique. This cell population was identified using phase-contrast microscopy and fractions with greater than 80% purity were pooled. RNA was extracted and gene expression was assessed using Affymetrix rat 230 2.0 whole rat genome microarrays. These studies were undertaken to determine the effects of advancing age on changes in gene expression in pachytene spermatocytes, a key, highly sensitive phase of spermatogenesis.

Results: Of the 31095 probe sets present on the array, 88% were expressed in young pachytene spermatocytes. 423 (1.4%) probe sets were expressed uniquely in the old: 242 (0.8%) that were expressed in the young were not present in the old. Of those expressed in both groups, aging caused altered expression of 607 (2%) probe sets with at least 2-fold change compared to young pachytene spermatocytes: 8% of these were upregulated while 92% were downregulated. Pathway analysis showed that a number of related genes were affected, including those involved in the oxidative stress response, such as SOD1, GSTM1, GSTM2, Mgst1 and Hifa.

Conclusion: In conclusion, the expression of many probe sets was altered in the aged pachytene spermatocytes suggesting that these cells, even though they are continually developing from stem cells, are quite different from those that are developing in the young tests; these differences may represent a response to the environment that they are in or an alteration in the stem cells in the aged rat tests.

Funding: This work was supported by a grant from CIHR.
Results: RA activity was found to be predominantly associated with premeiotic germ cells and was unevenly distributed throughout the seminiferous tubules. Additionally, β-galactosidase activity in premeiotic germ cells colocalized with STRA8 immunoreactivity and was induced with exogenous RA treatment. Treatment with inhibitors specific to the CYP26 family of RA degrading enzymes resulted in increased numbers of germ cells with β-galactosidase activity and STRA8 immunoreactivity and an increase in the expression of genes associated with differentiation. These results show that the action of RA in a subset of premeiotic germ cells leads to irregular initiation of differentiation throughout the neonatal testis and RA availability may be mediated through the action of CYP26 enzymes. Thus the presence of RA, possibly determined by CYP26 activity, appears to be a driving factor in the initiation of asynchronous sperm development.

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EFFECT OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) REPLACEMENT ON GONADOTROPIN RELEASING HORMONE-ANTAGONIST (GnRH-A) INDUCED MALE GERM CELL APOPTOSIS IN IGFBP-3 KNOCKOUT MICE

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Introduction: Apoptosis in male germ cells can be induced by GnRH antagonist (GnRH-A) in rodents and primates. We have recently shown that intratesticular injection of IGFBP-3 peptide can also trigger male germ cell apoptosis in the rat and IGFBP-3 knockout (KO) mice are resistant to GnRH-A induced testicular germ cell apoptosis.

Objectives: To further study the role of IGFBP-3 in male germ cell apoptosis, we examined the incidence of germ cell apoptosis in GnRH-A-treated IGFBP-3 knockout mice with or without IGFBP-3 peptide replacement.

Methods: Adult (38–43 week-old) male wild type and IGFBP-3 KO mice were studied. Groups of WT and IGFBP-3 KO mice were treated with a single sc injection of vehicle or GnRH-A (acryline 20 mg/kg BW). An additional group of GnRH-A-treated IGFBP-3 KO mice received an intratesticular injection of a synthetic IGFBP-3 peptide at day 13 and 14 post-GnRH-A. Animals were sacrificed at day 15. TUNEL was used to detect apoptotic germ cells. IGFBP-3 expression was demonstrated by western blotting (WB).

Results: Compared with wild type animals, IGFBP-3 knockout mice demonstrated significant resistance to GnRH-A-induced germ cell apoptosis. Local replacement of IGFBP-3 peptide into the testis was able to sensitize germ cells to apoptosis triggered by GnRH-A treatment. Exogenous IGFBP-3 was detected in the mitochondrial fractions of IGFBP-3 KO animals by WB after intratesticular injection of IGFBP-3 peptide.

Conclusion: IGFBP-3 plays an important role in male germ cell apoptosis. Mitochondrial enrichment of IGFBP-3 in the testes can be achieved by local injection of the IGFBP-3 peptide.

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CHARACTERIZATION OF A NOVEL TEKTIN MEMBER, TEKT5 IN MOUSE SPERM

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(Presented By: Wenlei Cao, MD, PhD)

Introduction and Objectives: Tektins are important components of flagella and alterations in the expression of or mutations in mouse tektins are correlated with defective sperm motility, a cause of male infertility. Our proteomic studies of sperm accessory structures previously identified a novel tektin, TEKT5, whose function is not known.

Methods: To understand the role of TEKT5 in mouse sperm better, we characterized the expression of the Tekt5 mouse gene and the presence of TEKT5 in spermatogenic cells and spermatozoa. A complete cDNA encoding the Tekt5 transcript was assembled following RT-PCR and 3' RACE and predicted that TEKT5 is a 62,730 Dalton protein with an unusual, long C-terminal tail. Tekt5 mRNA was highly expressed during late stages of spermiogenesis.

Results: Among examined tissues, Tekt5 mRNA was only present in testis and brain and quantitative RT-PCR showed that the expression level of mRNA in testis was 6.8-fold higher than that of brain. At the protein level, TEKT5 was present in sperm and was enriched in the accessory structures of flagella. Immunofluorescence confirmed that TEKT5 was localized throughout the complete sperm tail in sperm accessory structures.

Conclusion: The expression pattern suggests that TEKT5 plays an important role in flagella formation during spermiogenesis as well as being implicated in sperm motility.

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TESTICULAR HYPERThERMIA DECREASES RNA HELICASE DDX4 EXPRESSION IN APOPTOTIC GERM CELLS

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Introduction: We and others have previously demonstrated that 1) testicular hyperthermia induced germ cell apoptosis mainly occurs at the early and late stages of the seminiferous epithelia cycle in rat testes; 2) germ cell specific RNA helicase DDX4 (Vasa or Mvh) is expressed in pachytene spermatocytes and round spermatids that are most susceptible to heat-induced apoptosis; 3) RNA helicase DDX4 is one of the major components of the chromatoid body in late spermatocytes and round spermatids; 4) DDX4 Knockout mice are infertile with spermatogenic arrest at zygotene spermatocyte stage; 5) Recent evidence suggests that the chromatoid body is involved in translational regulation in the microRNA pathways of germ cell development. Thus, we hypothesize that increased testicular temperature may alter DDX4 expression and in turn interfere the microRNA pathways leading to germ cell apoptosis.

Objectives: To determine the role of DDX4 (a germ cell specific evolutionarily conserved RNA Helicase) in the regulatory mechanism of heat induced germ cell apoptosis in rat testis.

Material and Methods: Groups of 5 rats were used as control or received mild testicular hyperthermia. Wet heat treatment was performed with submerging rat scrotal containing testes in water bath at 43°C for 15 minutes. Testes samples were obtained at 30 minutes, 2 hours, 4 hours, 6 hours, and 12 hours after heat exposure. TUNEL Assay detected germ cell apoptosis. Western Blot and immunohistochemistry assessed DDX4 expression.

Results: DDX4 is specifically localized in pachytene spermatocytes and round spermatids in adult rats. DDX4 is one of the components of the chromatoid body. Decreased DDX4 expression in heat susceptible germ cells at early and late stages was observed as early as 2 hours preceding the DNA fragmentation in apoptotic germ cells occurring at 6 hours after heat exposure.

Conclusion: 1) Decreased testicular specific DDX4 expression disrupting RNA processing in translational regulation contributes to heat-induced germ cell apoptosis; 2) DDX4 is an early germ cell specific responder to heat stress in rat testis; 3) DDX4 may be a candidate target for male contraceptive development.
SPECIFIC ACVR1 GENE BY TAR DNA BINDING PROTEIN OF 43 KD (TDP-43)

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(Presented By: Avin Lalmansingh, PhD)

Introduction and Objectives: Spermatogenesis requires selective expression of genes at appropriate stages during germ cell development. TAR DNA binding protein of 43kDa (TDP-43) is an RNA Recognition Motif (RRM)-containing protein which functions in transcriptional repression. Here we show TDP-43 regulates the round spermatid-specific acrv1 gene. TDP-43 binds to TG sequences in DNA via an N-terminal RRM.

Methods: Using a plasmid-based Chromatin Immunoprecipitation (ChIP) system, we established TDP-43 binding to TGTGTG sequences in the acrv1 promoter. ChIPs of the endogenous acrv1 gene in germ cells showed TDP-43 binding to the promoter in its "off" and "on" states in spermatocytes and round spermatids, respectively. In contrast, RNA Poly II and active epigenetic marks H3K4me3 and H3K9ac showed highest enrichment at promoter in round spermatids. Using GAL4 strategy we next characterized TDP-43 repressor function in spermatocytic GC-2 cells. Transfections showed TDP-43 repressed acrv1 reporter activity when artificially recruited to promoter. The N-terminal 200 region including RRM1 was sufficient for repression whereas the C-terminal region (191-413) containing RRM2 and Gly-domain was not.

Results: These results reveal a novel function of the N-terminal RRM region in repression. The repressive core-regulators associated with TDP-43 are unknown. Since H3K4ac mark is enriched in the "on" state we asked whether histone deacetylases (HDACs) contribute to TDP mediated repression. Treatment of GC-2 cells with HDAC inhibitors Trichostatin A or Sodium Butyrate had no effect on acrv1 reporter activity, indicating HDAC independence. To begin to determine a role for post-translational modifications, we treated transfected GC-2 cells with proteasome inhibitor MG132 or phosphatase inhibitor Okadaic Acid. Both treatments relieved repression, suggesting that accumulation of ubiquitinated and phosphorylated forms of TDP-43 abrogates its repressive function.

Conclusion: Results suggest that ubiquitination and phosphorylation of TDP-43 likely serve as developmental switches mediating co-regulator associations, epigenetic changes and temporal control of target gene expression during spermatogenesis.

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MOUSE TESTICULAR DAMAGE IN CONTINUOUS AND INTERTMINTENT HYPOXIA

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(Presented By: Eduardo Bustos-Obregon, MSc, MD)

Introduction and Objectives: Hypoxia involves neoangiogenesis stimulated by low tissular oxygen supply with consequent intraescrotal increased temperature. In addition hypoxia is a stress factor leading to overproduction of reactive oxygen species (ROS). This work analyses the effect of hypoxia in CF-1 mice reproductive parameters. Animals were exposed to simulated hypoxia of 4,200 masl in a hypobaric chamber, for 33,2 days (complete spermatogenesis duration in mice), both of continuous (CH) and intermittent (IH) hypoxia. Intermittency was of 4 days hypoxia 4 days normoxia (500 masl) mimicking the schedule of copper mining workers in the North of Chile.

Methods: The anti-inflammatory agent Ibuprofen was administered to a group of mice. Melatonin was administrated to another group to evaluate its protective testicular action as a potent ROS scavenger. Animals in both CH and IH exposure were compared to normoxic non treated controls. Parameters analyzed were hematological response to hypoxia and testicular damage (Immunohistochemistry and morphometry).

Results: Results indicated that in hypoxia there is hematological response with increase in hematocrite and reticulocitosis. Hif-1 alfa immunohistochemistry reveals an increased expression in the seminiferous tubules, and also HSP 70, indicative of cell damage. In CH morphometric analysis of seminiferous tubules indicated decreased tubular diameter and lumen. Histopathological analysis of seminiferous tubules revealed increased histological alterations (vacuolization, basal membrane foldings, etc). Quantification and diameter of testicular blood vessels increased in both hypoxic models. Therefore, hypoxia exposure damages the testis, more in CH than in IH, suggesting that alternancy of normoxic periods permit compensation of the effects of hypoxia. Melatonin exerts protective action in the testis, decreasing Hsp 70 expression and histopathological tubular alterations. Ibuprofen exerts a protective effect on the same parameters both in IH and CH. Also, HIF-1 alfa expression was decreased as well as testicular blood vessels count and diameter.

Conclusion: In conclusion, intervention upon ROS (melatonin) and neoangiogenesis (antiflogistic agent) protect against hypoxic testicular damage.
Introduction and Objectives: Quercetin (QR) is a strong antioxidant and has been shown to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced diabetes in animal models. Antioxidants have significant effects on spermatogenesis, sperm biology and oxidative stress, and changes in antioxidant capacity are considered to be involved in the pathogenesis of chronic diabetes mellitus. The present study, aim to examine the influence of quercetin on spermatogenesis in STZ-induced diabetes in male Wistar rats.

Methods: Animals (n = 50) were allocated into five groups: Group 1: Control rats given 0.5ml of 20% glycerol in 0.9% normal saline. Group 2: Control rats given buffer (pH4.0). Group 3: diabetic controls. Group 4: rats given Quercetin (QR) 15mg/kg/day (i.p.). Group 5: STZ + Quercetin rats. Animals were kept in standard conditions. At the end of the experiment (28th day), blood samples were taken for determination of testosterone, total serum testosterone increased significantly in QR-treated diabetic rats in comparison with controls (P < 0.05).

Results: Sperm numbers, percentages of sperm viability and motility and total serum testosterone increased significantly in QR-treated diabetic rats (P < 0.05) compared with control groups. In histopathology, degeneration and inflammation in testes cells associated with diabetes were improved and testes weights in the QR-treated diabetic group decreased significantly in comparison with controls (P < 0.05).

Conclusion: We conclude that QR has significant beneficial effects on the sperm viability, motility and serum total testosterone and could be effective for maintaining healthy sperm parameters and male reproductive function in diabetic rats.

Conclusion: Because miRNAs—including let-7 family members critical for germine maintenance—undergo post-translational modification (Reid JG et al., 2008), we quantified the percentage of reads with 1-2 mismatches, revealing a developmental trend to this modification. We identified several putative novel testis-abundant miRNAs whose developmental expression suggests may play a role in early male germ cell development.

Funding: These studies were supported in part by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreements U54-HD07495 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research and U01-HD60496 as part of the Cooperative Program in Male Contraception (to M.M.M.) and T32–HD07165 (to G.M.B.).

Introduction and Objectives: The developmental expression of ACRV1 in human and mice. We detected expression of miRNAs encoded on the X chromosome, specifically in the human testis and in round spermatids, indicated ACRV1 is a potential male contraceptive target for humans.

Funding: National Natural Science Foundation in China (30700824); “973”Plan in China (2008CB517412); Chinese Higher Education Doctoral Programme Fund (200800010106); Guangdong Provincial Health Research Project in China (A2008634).

Introduction and Objectives: At present it is not clear if male fertility is affected by intermittent hypobaric hypoxia (IHH). This is an important issue since a large human population works over 3000 masl.
**Methods:** This study analyzes epididymal sperm, in adult Sprague Dawley rats after five cycles of IHH (7 days exposure to 4200 masl in a hypobaric chamber / 7 days at 500 masl). The animals were separated into groups of 8, one group was exposed to hypoxia (7 days), and the others were to IHH for one to five cycles. Controls (500 masl) were examined at the beginning and at the end of the 70 experimental days. Damage in hypoxic tissues is mostly due to reactive oxygen species (ROS) overproduction. For this reason a duplicate set of rats treated with melatonin (ROS scavenger) was also examined, as were their controls, injected with 0.03% ethanol (melatonin solvent). Epididymal sperm parameters, were evaluated.

**Results:** Damage caused by IHH increases with time. Sperm counts drop, while sperm chromatin swelling, DNA instability (metachromasia with acridine orange epifluorescence) and comet (+) tests increase. Melatonin counteracts all this noxious effects, possibly due to its high efficiency as a ROS scavenger.

**Conclusion:** In conclusion, IHH exposure damages sperm quality and possibly affects male reproductive function.

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**ROLE OF GHERLIN AND LEPTIN IN MALE REPRODUCTION**

Carolina Jorgez, PhD, Shannon Whirledge, PhD, Aysegul Sahin, BS, Roy Smith, PhD and Dolores Lamb, PhD

**Introduction and Objectives:** Male fertility requires the integration of the hypothalamic-pituitary-gonadal axis pathways and networks regulating body homeostasis for proper testicular function. In addition to the gonadotropins and sex steroid hormones, the hormones regulating energy balance and metabolism, ghrelin (Ghs) and its antagonist partner, leptin are implicated in male reproduction. Leptin, Ghs and its receptor GHSR are expressed in human and rodent testis where they have been examined individually but not their interplay. Ghs inhibits expression of several steroidogenic proteins and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile, with small testes and low circulating testosterone. Our hypothesis is that Ghs and testosterone secretion. Leptin-deficient mice (ob/ob) are infertile.
Introduction and Objectives: Developmental genitourinary (GU) birth defects are associated with mortality and morbidity in adults. Complex diseases such as testicular cancer and male infertility are associated with developmental abnormalities of the male GU tract, including cryptorchidism and hypospadias. Cryptorchidism results from failure of testis descent. It occurs in 2% of full-term newborn boys and results in impaired spermatogenesis. Hypospadias is a midline fusion defect of the male ventral urethra and occurs in nearly 1 in 100 live male births. The etiologies of these congenital GU birth defects are poorly understood and likely involve multiple genetic and environmental factors. Detection of these congenital aberrations typically involves karyotyping which has a limited resolution and is unable to detect submicroscopic, clinically significant chromosomal rearrangements. We hypothesize that high resolution array CGH will allow the detection of subtle chromosome aberrations in patients presenting with both hypospadias and cryptorchidism. Furthermore, analysis of these patients will reveal regional clusters and will likely involve genes critical for GU tract development.

Materials and Methods: We used genome-wide comparative genomic hybridization microarray (CGH array) analysis of chromosomal defects in children with congenital genitourinary defects including combined hypospadias/cryptorchidism. Sex-matched genomic DNA from men of proven fertility served as a reference in these studies. Genomic DNA was analyzed by comparative genomic hybridization array (aCGH) using 720K NimbleGen arrays (Roche) and analyzed using Nexus Copy Number software (BioDiscovery). FISH and/or QPCR were employed to validate putative regional gains or losses distinct from common copy number variants (CNV) found throughout the genome.

Results: Patients with both hypospadias and cryptorchidism displayed distinct chromosomal regions of chromosome duplications or deletions; including the regions: 5p13, 7q34, 9p11, 11q12, 15q11 and 22q11, which encode genes with putative roles in GU tract development and fertility. Some of these regions contained CNVs, therefore further analysis is needed to identify regional clusters associated with combined hypospadias/cryptorchidism defects.

Conclusion: Array CGH represents an accurate high-resolution method to identify clinically significant chromosomal aberrations associated with congenital GU tract defects.

CDNA MUTATIONS IN ZPB1 ASSOCIATED WITH TERATOZOOSPERMIA IN INFERTILE MEN
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Introduction and Objectives: Male infertility is a global health problem of multifactorial etiology, affecting nearly 7% of men. It is estimated that genetic factors account for at least 50% of cases of male infertility. Teratozoospermia is a common semen abnormality condition associated with male infertility. It is defined as an abnormal morphology with less than 4% of the normal spermatozoal content. However, genetic defects that cause teratozoospermia are mainly unknown in the majority of patients. In the present study, we investigated the role of STYX (phosphoserine/threonine/tyrosine-binding protein) in teratozoospermia in humans. Previous studies demonstrated that male mice with the disrupted ZPB protein were unable to fertilize oocytes due to abnormal spermatozoal morphology and forward sperm motility. Electronic microscopy of spermatozoa showed abnormal ultrastructure, round-headed globozoospermia-like morphology. The ZPB is a structural protein, predominantly expressed in spermatozoal acrosome, and plays important role in acrosome reaction, binding to oocyte zona pellucida, and ultimately in oocyte penetration. Therefore, it was suggested that abnormal morphology affects acrosome compaction and oocyte penetration that results in inability to fertilize oocytes.

Methods: To study association between ZPB1 mutations and teratozoospermia, we selected 192 cDNA samples from infertile males with abnormal semen morphology and 100 normozoospermic controls. Following RT-PCR and cDNA sequencing have been performed with those samples.

Results: Preliminary, we identified several novel heterozygous missense and splicing alterations in cDNA samples from infertile patients with teratozoospermia. Analysis of the dbSNP database showed that none of these nucleotide changes are listed as known polymorphisms. The observed cDNA defects will be confirmed in the genomic DNA.

Conclusion: Our preliminary data suggest that mutations in ZPB1 may contribute to a significant fraction of teratozoospermia in infertile men. Further genetic studies are in progress.

Funding: This study was supported in part by the National Institutes of Health Infertility Center (P01HD36289) and US4 the Specialized Cooperative Centers Program in Reproduction and Infertility Research to DJL and MMM.
CLONE AND ANALYSIS OF THE HOMOLOGOUS SEQUENCES OF HUMAN TESTIS DEVELOPMENT RELATED GENE 1 IN DIFFERENT SPECIES

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(Presented By: Tang Yuxin MD)

Introduction: Spermatogenesis is a highly coordinated physiological process that involves proper functions of thousands of gene products. In our department we employed Bioinformatics analysis coupled with reverse transcription polymerase chain reaction (RT-PCR) to clone a novel human testis specific gene named Human Testis Development Related Gene 1, TDRG1 (GenBank Accession Number: X6DQ 168992). The expression of TDRG1 was exclusively detected in human testis but not in any other non-reproductive tissues. Intriguingly, TDRG1 exhibited the highest expression level at human puberty testicular tissue, with but no expression in human testis in the embryonic stage and decreasing levels of expression with aging, indicating TDRG1 may have a role in human spermatogenesis and fertilization.

Aim: To analyze and clone the homologous sequences of TDRG1 in different species and to explore the potential animal models for further researching the function of this gene.

Methods: The nucleotide sequence of TDRG1 was used as a query sequence to search for the mouse, rat, chimpanzee and rhesus macaque genome databases by BLASTN program to obtain the homologous sequences in these species. The homologous sequences of TDRG1 were then confirmed by RT-PCR. Immunohistochemistry was used to determine the expression of homologous proteins in the testes of these species by specific anti-TDRG1 monoclonal antibody.

Results: Bioinformatics analysis indicated that no homologous sequences of TDRG1 were found in the mouse and rat genome databases. However, highly homologous sequences were detected in chimpanzee and rhesus macaque genome databases, the percentage of gene homology were 98% and 90% respectively. The homologous sequence in rhesus macaque testicle was confirmed by RT-PCR. Immunohistochemistry results demonstrated that anti-TDRG1 monoclonal antibody specifically recognized the homologous protein in rhesus macaque testicle. Whereas, RT-PCR coupled with Immunohistochemistry provided convincing evidences that there was no homologous gene expressed in mouse and rat testes.

Conclusion: No gene sequence shows to be homologous with TDRG1 in mouse and rat testes. However, the homologous genes were found in rhesus macaque and chimpanzee testes, suggesting that TDRG1 may exclusively express in primate. This study also provides potential candidates for developing TDRG1 transgenic or knockout animal models.

Funding: National Natural Science Foundation of China (30672090).

NOVEL PROTAMINE2 (PRM2) MUTATION AND TRANSITIONAL PROTEIN (TP) GENE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) AFFECT SPERM DNA INTEGRITY AND MOTILITY IN INFERTILE MEN

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(Presented By: Marta Olszewska, MSc)

Introduction and Objectives: Reciprocal chromosomal translocations (RCT) are the most common structural rearrangements in humans. RCT carriers produce all types of genetically unbalanced spermatozoa (alternate, adjacent I, adjacent II, 3:1, 4:0) which are result of chromosomes' segregation after forming quadrivalent in meiosis. The pattern of meiotic segregation is determined by the chromosome type involved in translocation, breakpoints localization, the length of the interstitial and translocated segments and by the localization and number of chiasmata. About 1% of RCT is associated with male infertility. Six different RCT carriers with reproductive failure: t(11;13), t(1;11), t(3;9), t(8;14), t(11;16) and t(7;18) were included in our analysis of meiotic segregation pattern and sperm DNA fragmentation. Four of them were normozoospermic, while carrier of t(5;9) had an asthenoteratozoospermia and carrier of t(11;13) an oligozoospermia.

Methods: To analyze meiotic segregation pattern a triple-colour FISH (fluorescent in situ hybridization; alpha satellite and subtelomeric probes, CytoCell UK) on 3,500 sperm cells in each case was performed. The sperm DNA fragmentation was detected by TUNEL labeling and flow cytometry.

Results: The frequencies of unbalanced spermatooza for alternate segregation ranged from 34% for t(7;18) to 57% for t(4;18) with a mean of 50%, for adjacent I: 29% for t(11;13) - 37% for t(1;11) with a mean of 33%, for adjacent II: 1% for t(3;9) - 18% for t(7;18) with a mean of 5.5% and for 3:1 : 5% for t(4;18) - 13% for t(7;18) with a mean of 9.5%. No results for 4:0 were found. For six male RCT carriers the percentage of DNA fragmentation varied between 5.66% for t(7;18) and 34.12% for t(4;18) with a mean of 15.85%.
Conclusion: Segregation pattern analysis of each new RCT case is important for genetic counseling especially in families with poorly collected pedigree data. Until now only two studies described the DNA fragmentation in sperm cells of RCT carriers and showed higher rate of the sperm DNA fragmentation comparing to controls which is similar to our results. Higher frequency of the sperm DNA fragmentation may also explain the nature of observed reproductive failures.

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Y CHROMOSOME MICRODELETIONS AND PARTIAL DELETIONS OF THE AZFc REGION IN NORTH INDIAN INFERTILE MEN
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(Presented By: Madhukar Dama, Master of Veterinary Sciences)

Introduction and Objectives: The present study was designed to assess for the first time the occurrence of Y chromosomal AZFc region partial deletions and AZF microdeletions in Indian men (Uttar Pradesh) and to correlate them with clinical parameters.

Methods: In a retrospective study, we analyzed 107 infertile men and 100 controls with normal spermatogenesis. AZFa, AZFb, AZFc and partial deletions within the AZFc region were analyzed by polymerase chain reaction (PCR) according to established protocols.

Results: No AZFa, AZFb or AZFc deletions were found in the control group. Four patients in the group of infertile men were found to have deletions as following: one AZFabc and three AZFc. The relative distribution of these patterns was different compared with that found in the other populations. Extension analysis confirmed that the deletions occurred according to the current pathogenic model. gr/gr deletions were found to be present in the patients (n = 6) but not controls as reported by previous studies.

Conclusion: These results suggest that the frequency and pattern of microdeletions of the Y chromosome in Uttar Pradesh men are lower than those found in other populations and raise questions on the risk of gr/gr deletions on spermatogenic failure.

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ANALYSIS AND SIGNIFICANCE OF Y CHROMOSOME TRANSCRIPTS IN HUMAN EJACULATED SPERM FROM NORMOZOOSPERMIC DONORS: COMPARISON WITH SPERMATOZOA FROM INFERTILE MEN
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(Presented By: Rajender Singh, PhD)

Introduction: The existence of a complex population of mRNA in human sperm is well documented but their role is not yet elucidated.

Objectives: To quantitatively microdissect the mRNA specifically originating from Y chromosome.

Methods: Polymerase chain reaction and expression profiling of mRNA obtained from spermatozoa of normal fertile men and comparison of the variation in the expression of the present transcripts in spermatozoa from infertile men.

Results: Of all the Y chromosome transcripts analyzed, five transcripts (HSFY1, HSFY2, XKRY, RBMY and CSPG4LY) were detected consistently in spermatozoal RNA from fertile men. The transcripts were detected variably in spermatozoal RNA of infertile men. Analysis is presented for presence and absence of these transcripts and quantitative variation in fertile individuals in comparison to fertile controls.

Conclusion: Detection of Y chromosome transcripts for the first time in spermatozoal mRNA suit suggests a possible role for these transcripts. Further the variation of expression in infertile individuals shows that the transcripts have definite role in maintaining normal fertility of male gametes.

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IDENTIFICATION OF COPY NUMBER VARIANTS ASSOCIATED TO GENITOURINARY BIRTH DEFECTS AND CHARACTERIZATION OF NOVEL KEY GENES IN HUMAN GENITAL DEVELOPMENT
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(Presented By: Shuo Han)
Two key actors in male external genitalia development. We also investigated to induce DM. Mice were sacrificed 8 weeks (Group 2, n=15) after alloxan. The remaining 15 of them were injected with alloxan (100 mg/kg body weight). Vamp7 gene expression was not affected, its protein levels increased in diabetic mice.

Results: Confirmed de novo duplication and deletion events were significantly associated with GU defects (P=1.02x10^-6) as compared to 15,931 non-GU patient controls on 1p36.33, 9p23p24 and 19q12-q13.11 for ambiguous genitalia and 10p14 and Xq28 for cryptorchidism and 12p13, 16p11.2 and 16q24.3 for hypospadias. Of note, a gain on Xq28 was found in 2 unrelated patients and encompassed a single gene: vesicle-associated membrane protein 7 (Vamp7). Using FISH, Xq28 gain was confirmed in patients and was considered as de novo event using DNA parental analysis. Vamp7 knockdown was performed in NTERA-2 cells that recapitulate the expression profile of endogenous markers of male sex determination and differentiation. Suppression of Vamp7 significantly increased the gene expression of Hoxa13 and its downstream target Fgf8, two key actors in male external genitalia development. We also investigated the effect of testosterone on the expression of Vamp7 in NTERA-2 cells as these GU defects may result from defective androgen signaling. While Vamp7 gene expression was not affected, its protein levels increased in presence of androgens.

Conclusion: Taken together, the identification of these significantly copy number variants will help to elucidate the molecular mechanisms underlying the pathogenesis of human genital development and define critical factors such as Vamp7 in male sexual development.

**MALE SEXUAL FUNCTION**

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**INFLAMMATION MAY PLAY AN IMPORTANT ROLE IN THE ERECTILE DYSFUNCTION ASSOCIATED WITH TYPE 1 DIABETES**

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(Presented By: Donghua Xie, MD, PhD)

Introduction and Objectives: Nuclear factor (NF)-kappaB, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) are important factors in inflammation. We sought to investigate the changes in these inflammatory factors in type 1 diabetes mellitus (DM) induced erectile dysfunction (ED) in mice.

Material and Methods: In total, 30 C57BL6 (Bl-6) mice were divided into 2 groups (n=15/group). Fifteen of these animals (Group 1) had no treatment. The remaining 15 of them were injected with alloxan (100mg/kg body weight) to induce DM. Mice were sacrificed 8 weeks (Group 2, n=15) after alloxan-induced DM. Corporal tissues were harvested and studied for vasoreactivity by isometric tension study; Level of cyclic guanosine monophosphate (cGMP) by enzyme immunoassay assay (ELISA); Levels of NF-κB components (p65, p50), COX-2, and iNOS were assessed by western blot analysis.

Results: Endothelium-dependent and endothelium-independent vasoreactivities, and cGMP were significantly decreased while p65, p50, COX-2, and iNOS were significantly increased in the corporal tissue of diabetic mice.

Conclusion: Inflammation may play an important role in the progression of erectile dysfunction associated with Type 1 diabetes.

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**CORRELATION OF SERUM TESTOSTERONE LEVELS IN MEN PRESENTING WITH ERECTILE DYSFUNCTION USING DIFFERENT VALIDATED QUESTIONNAIRES**

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(Presented By: Osama Mohamed, MD)

Introduction and Objectives: Serum testosterone levels have been shown to correlate with the Androgen Deficiency in the Aging Male (ADAM) questionnaire. However, there is sparse data on the correlation between serum testosterone levels and other questionnaires offered to men who present for erectile dysfunction (ED). These questionnaires include the International Index for Erection Function (IIIEF-15), International Prostate Symptom Score (IPSS), Ewport Sleepiness Scale (ESS) and more recently the quantitative ADAM (q-ADAM) questionnaire.

Methods: Men presenting with ED were asked to complete the IIEF-15, the IPSS, the ESS, and the qADAM to assess for ED, BPH, obstructive sleep apnea, and signs of hypogonadism, respectively. Serum testosterone levels were collected at the time the questionnaires were completed.

Results: 54 patients participated in this study. The mean age was 48.8 years (+/-13) and mean BMI was 32.4. Associated co-morbidities included hypertension (33.9%), diabetes mellitus (18.8 %) and dyslipidemia (33.9 %). There was a significant association between serum testosterone levels and the qADAM questionnaire (odds ratio = 8.232, p<0.004). Moreover there was a significant association between hypogonadal men and a positive finding on the Ewport Sleepiness Scale (odds ratio = 4.49,p =0.034). There were no significant association between serum testosterone levels and the IIEF-15 or the IPSS questionnaires (p>0.05). However there was a strong correlation between qADAM scores and IIEF scores (Pearson correlation= 0.595, p<0.01).

Conclusion: While serum testosterone levels appear to correlate with qADAM and ESS questionnaires, serum testosterone does not appear to be correlated with the IIEF-15 or IPSS questionnaires. Finally, qADAM and IIEF-15 questionnaires correlate with one another suggesting that hypogonadism and ED symptoms are related.

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**EFFECT OF MUCUNA PRURIENS (LINN.) ON SEXUAL BEHAVIOR AND SPERM PARAMETERS IN STREPTOZOTOCIN INDUCED DIABETIC MALE RATS**

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(Presented By: Seppan Prakash, PhD)

Objective: To analyze the efficacy of the Mucuna pruriens (M. pruriens) on male sexual behavior in long-term hyperglycemic male rats.

Methods: Animals (Rattus norvegicus) were divided as group I – control, group II – Diabetes (induced by single intramuscular dose of streptozotocin (STZ) 60 mg/kg of body weight (b.w.) in 0.1 M citrate buffer), group III – Diabetes and administered with ethanolic extract of M. pruriens seed extract 200 mg/kg b.w., group IV – Diabetes and administered with 5 mg/kg b.w. of Sildenafil citrate (SC), group V – received 200 mg/kg b.w. of M. pruriens seed extract and group VI – received 5mg/kg b.w. of SC. The M. pruriens and the SC were administered orally, once daily for a period of 60 days, accordingly. At the end of 60 days, all the animals were subjected to mating behavior analyses and libido and test of potency. The sperm were collected from caudal portion of the epididymis was subjected to various analyses.

Results: Results showed significant reduction in sexual behavior and sperm parameters in group II. Daily sperm production (DSP) and hormone levels were significantly reduced in group II. The diabetic animals administered with seed extract of M. pruriens (group III) showed significant improvement in sexual behavior, libido, potency, sperm parameters, DSP, hormonal levels when compared to group II.

Conclusion: From the present study we conclude that ethanolic seed extract of M. pruriens has the potential to improve male sexual behavior along with androgenic and anti-diabetic effects in the STZ induced diabetic male rats. These results form the scientific evidence in supporting the claim made in Indian traditional system of medicine that the M. pruriens is clinically useful as a sexual invigorator in diabetic men.
WEIGHT LOSS IMPROVES SEXUAL AND LOWER URINARY TRACT FUNCTION IN OBESE DIABETIC MEN: EFFECTS OF ENERGY RESTRICTION, NUTRITIONAL QUALITY AND TIME COURSE
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Introduction and Objectives: Obesity and type 2 diabetes mellitus (T2DM) are associated with sexual and lower urinary tract dysfunction in men. We determined the effect, on these symptoms, of 8 weeks of rapid diet induced weight loss and 44 weeks of weight maintenance, and the possible mechanisms involved.

Methods: Obese men (n=31) (BMI >30 kg/m2, waist circumference (WC) ≥102 cm, mean age 59.7yrs) with uncomplicated diet or metformin treated T2DM were recruited by advertisement. None had previously sought treatment for sexual or lower urinary tract symptoms. Weight loss was induced over 8 weeks with either a low calorie (-1000kcal/day) meal diet (n=12) that reduced energy intake by 600kcal. Thereafter both groups continued on the CSIRO diet. Weight, WC, International Index of Erectile Function (IIEF) score, Sexual Desire Inventory (SDI) score, International Prostate Symptom Scale (IPSS) score, plasma testosterone (T), metabolic parameters, BP, and brachial arterial flow-mediated dilatation (FMD) using MRI, were measured at baseline, 8, and 52 weeks. Plasma hsCRP, myeloperoxidase, interleukin-6 (IL-6), sE-selectin, and nitrate at baseline and 8 weeks. Data were analyzed by 2 way repeated measures ANOVA.

Results: At baseline the mean (± SE) scores for IIEF (8.1±1.4 and 11.6±2.2), SDI (44.4±5.4 and 51.9±6.3) and IPSS (6.3±1.1 and 9.0±1.8), in the LCD and CSIRO groups respectively were not significantly different. Subject retention was 100% at 8 weeks and ~50% at 52 weeks. At 8 weeks, weight and WC decreased by ~10% and ~5% in the LCD and CSIRO groups. The IIEF and SDI scores, and plasma T increased, and IPSS scores and sE-selectin decreased, similarly on both diets. Plasma IL-6 and hsCRP levels decreased in the CSIRO group. By 52 weeks, weight and WC, were similarly decreased in each dietary group. Further increases in IIEF, SDI and FMD and decreases in IPSS occurred in each group. Plasma T was unchanged. BP and metabolic parameters improved, there were effects of diet, time, and diet x time interactions.

Conclusion: Diet-induced weight loss improved sexual, urinary and endothelial function in obese diabetic men progressively over 12 months. The initial lesser weight loss in the CSIRO diet group may have been offset by the improved inflammatory profile. Further improvements in both dietary groups at 1 year, and without further weight loss in the LCD group, suggests that both caloric restriction and nutritional quality are important.

CLINICAL CHARACTERISTICS OF THE PATIENTS WITH LIFELONG PREMATURE EJACULATION OF LESS THAN 1 MINUTE IELT
Joon Yong Kim, Professor, MD, Byung Moo Philip Kim and Si Jin Paul Kim Philip and Paul Medical Institute

Introduction and Objectives: At present, diverse discussion have been made on the definitions, causes and clinical opinions of premature ejaculation. This study aims to analyze sexual activity patterns and the results of clinical laboratory studies of the patients with lifelong premature ejaculation of less than 1 minute IELT.

Method: The subjects were those who ejaculated within one minute after insertion, did not have any other disease history, and no other sexual dysfunction except for premature ejaculation. In this study, their sexual activity patterns were researched and physical examination, penile sensitivity test, blood test and prostatitis test were conducted on them.

Results: The number of subjects was 75 and their average age was 38.9 years. Among them, 36 were married and 39 were single. 21 of them reported ejaculation within 10 seconds after insertion. The threshold of biothesiometry was 5.3±1. 6. 25(33.3 %) of them showed less than 4 threshold. All were normal in the prostatitis test and the PSA was 0.9±0.5 ng/ml, which was in the normal scope. Their thyroid hormone levels were T3 1.1±0.2ng/ml, and T4 8.4±1.6μ/dl, respectively. 3(4%) of them showed hypothyroidism while 1(1.3%) of them showed hyperthyroidism. The level of total testosterone and free testosterone was 522±211ng/dl and 13.5±5.6 pg/ml, respectively. 5(6.6%) of them had increased secretion of testosterone, 1 of which had increased secretion of LH. One man had deficiency in testosterone but their LH and FSH level was in normal scope. Their leptine level was 3.9 ± 4.2 ng/mL and 81.8% of the subjects showed prolongation of the ejaculatory latency after using anesthetic cream. As for the question about the reason of their premature ejaculation, 30% responded that they had no idea; 50% responded that it was due to penile hypersensitivity and 10% responded to psychological reason.

Conclusion: In case of consulting the patients with lifelong premature ejaculation of less than 1 minute IELT, it may be considered to take thyroid hormone test, testosterone test and biothesiometry as a selective test. As for treatment, along with the generally-used drug therapy such as SSRI's and behavioral therapy, it is recommended to use penile sensitivity approach as a method to prolong the ejaculatory latency.

SALVAGE PENILE CURVATURE CORRECTION SURGERY
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Introduction and Objectives: It is believed that coarser suture materials should be used to provide sufficient tenacity in penile tunical surgery. We report our 15-year experience of using finer sutures in a salvaging operation in 31 patients who underwent prior curvature correction elsewhere with coarser sutures.

Methods: Suture materials used in prior surgeries in these patients were 2-0/3-0 nylon sutures. In this series, all 31 patients underwent a modified Nesbit procedure at the level of the collagen bundles using finer sutures. Prior to July 1998, 10 men underwent salvage surgery using 4-0 polyglactin sutures. Thereafter, we have adapted 6-0 nylon sutures for another 21 patients. These were categorized into the polyglactin and nylon groups respectively. Overall, 29 patients were available for a follow-up while using the abridged five-item version of the International Index of Erectile Function (IIEF-5) scoring system with 21 patients in the nylon group. We have found cavernosography a practical and reliable method to objectively assess penile morphology in these patients.

Results: The penile morphology both subjectively and objectively was excellent in all patients, except for one in each group. Erectile function restoration showed a trend of satisfaction in the polyglactin group and based on IIEF-5 was significantly improved in the nylon group (14.2±3.6 vs. 21.9±2.1, n=20, p<0.001).

Conclusion: We may suggest that in penile tunical surgery, fine sutures such as 6-0 nylon may be an ideal suture material for resulting better penile morphology and functional outcomes since it has been sustainable in salvaging tunical surgery.
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**SALVAGING PENILE VENOUS STRIPPING SURGERY**
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(Presented By: Heng-Shuen Chen, MD, PhD)

**Introduction and Objectives:** Disappointing functional outcome and penile deformity are major concerns of penile venous surgery. Consequently, it has been abandoned by most urologists. To explore whether penile deformity is correctable and erectile function can be improved, we report our experience in patients who had undergone surgery elsewhere.

**Methods:** From 1996 to 2008, 16 consecutive patients sought our assistance because of poorer erectile capability or/and penile deformity from previous venous surgery elsewhere. The abridged five-item version of the International Index of Erectile Function (IIEF-5) was used to score the patients when it became available in 1998. Accordingly, 3 and 13 patients were categorized into the non-IIEF and IIEF groups respectively. A median longitudinal pubic incision and a circumferential or semi-circumcision were made to relieve the fibrotic tissues for accessing the deep dorsal veins which were stripped thoroughly and ligated with 6-0 nylon sutures. The cavernosal veins were managed in a similar manner. The para-arterial veins were ligated only segmentally. Finally, the wound was approximated layer by layer while an assistant consistently stretched the penile shaft.

**Results:** The operation time was from 5.2 to 8.5 hours. The follow-up period ranged from 0.6-23.0 years. Overall all patients reported satisfactory penile morphology postoperatively. In the IIEF group, difference in pre-operative and post-operative scores was significant (P < 0.001). In the non-IIEF group two out of the 3 patients reported natural coitus.

**Conclusion:** This series of salvaging venous surgeries, though technically challenging, are helpful in correcting penile deformity and restoring erectile function in some patients who had poorer outcomes from prior venous surgeries.

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**CHRONIC USE OF A SHORT-ACTING PDE-5 INHIBITOR TO RESTORE ERECTION AND EJACULATION BEFORE IVF IN A CASE WITH ACQUIRED HYPOGONADISM AND HYPERPROLACTINEMIA**
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(Presented By: Juan Correa-Perez, PhD)

**Introduction and Objectives:** The use of PDE-5 inhibitors (PDE-5i) is considered as first-line therapy for the treatment erectile dysfunction (ED). Recent studies have suggested chronic PDE-5i use in patients that do not respond to on-demand regimens, or individuals with metabolic conditions (i.e., diabetes, metabolic syndrome). In this study, we describe the chronic use of short-acting PDE-5i’s to reestablish erection and ejaculation in a man with acquired hypogonadism and hyperprolactinemia before initiation of an IVF cycle.

**Methods:** A 42-year-old man presented to our clinic for male work-up before an IVF cycle. The patients’ major complaint consisted of erectile dysfunction and hypogonadism. In addition, hyperprolactinemia, dyslipidemia and elevated glycosylated hemoglobin (HbA1c) were also present as contributing factors. Treatment with bromocriptine was initiated to normalize prolactin levels. The patient was then started on sildenafil citrate (50 mg/day) after normalization of prolactin levels. The strategy was to take the PDE-5i daily until the establishment of an erection without attempting intercourse or ejaculation. The next step was to attempt intercourse and ejaculation after determining the effective dose. The PDE-5i dose was titrated as necessary.

**Results:** The patient reported achievement of an initial erection after 3 d of PDE-5i intake. The dose was titrated to ‘100 mg, followed by a sustained erection and ejaculation during intercourse. A semen specimen was produced 7 d later (Frozen). A second semen specimen was produced on the day of oocyte retrieval and used for split IVF/ICS with an overall fertilization rate of 60%. Embryo transfer was performed on Day 5 (3 embryos), resulting in a singleton pregnancy.

**Conclusion:** Chronic Intake of short-acting PDE-5i was well tolerated by the patient. Previous use of on-demand PDE-5i’s by the patient had proven ineffective. Establishment of an initial erection was achieved even in the presence of low testosterone levels (< 100 ng/dl) and symptoms consistent with metabolic syndrome. Chronic use of PDE-5i’s may present a safe alternative to reestablish erection and ejaculation in ED cases of an organic etiology, which otherwise may not respond to on-demand regimens.

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**NEW PENILE AUGMENTATION SURGERY TECHNIQUE BY INJECTING MICRONIZED HUMAN CELL-FREE DERMAL TISSUES INTO SUBCUTANEOUS TISSUE**
Joon Yong Kim, Professor, MD, Byung Moo Philip Kim and Si Jin Paul Kim
Philip and Paul Medical Institute
(Presented By: Joon Yong Kim, Professor, MD)

**Introduction and Objectives:** Penile augmentation surgery with tissue grafts through incision has been performed so far. Surgery can also be carried out through the injection of chemical filler or fat. However, the incision method has shortcomings including a long recovery time and a complicated process, while the injection method has the restrictions of instability of materials, a high recurrence rate of absorption and distortion of shape. We have performed penile augmentation surgery by injecting acellular allogenic dermal tissue.

**Material and Methods:** The surgery was done for men with a small penis complex. Acellogenous collagen does not require skin testing. According to the size of the penis and the augmentation size the patient desired, about 3–6cc of dried acellular particulate dermal matrix (the granule of the tissue was 500–1000µm) combined with 1.5–1.8cc of lidocaine and 0.3cc of gentamicin per 1cc of the tissue were injected. After local anesthesia, it was injected into the subcutaneous tissues of the whole shaft except for the 5–7 o'clock direction on the ventral side.

**Results:** Retrospective investigation was done for this study with 111 cases from December 2007 to August 2009. The average age was 45.2 years and the average surgery time was 20 minutes. 4cc of acellular micronized dermal tissues on average were used for a one-time injection. There was an augmentation effect of 3.1cm on average around the shaft of the penis. 2 case of local skin necrosis was reported. There was little nodule of the injected tissues. Patients return to normal life immediately after surgery.

**Conclusion:** This surgical method has several advantages compared to existing augmentation surgery. It does not require incision, takes a short operation time, has rapid recovery time and few side effects. Therefore, for the men who experienced difficulty with the preexisting techniques, for example, those who had physical health problems, or were of an older age or were on special medications, this surgical method could be recommended. In particular, in the case of re-augmentation surgery after the first augmentation surgery and in the case that required correction due to penile distortion, it is regarded as the ideal method.

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**Before**

**After**
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**PENILE VEINS ARE THE DETERMINING CONTRIBUTOR FOR ERECTION: THE HEMODYNAMIC EVIDENCE FROM THE STUDY IN DEFROSTED HUMAN CADAVERS**

Geng-Long Hsu, MD, Yi-Ping Huang, PhD, Meng-Hung Tsai, PhD, Kuo-Liang Chen, MD, Chao-Hsiang Chang, MD and His-Chin Wu, MD

1 China Medical University; 2 China Medical University Hospital

(Presented By: Geng-Long Hsu, MD)

**Introduction and Objectives:** Penile venous surgery for treating erectile dysfunction (ED) is currently considered experimentally and the venous factor is not included as a contributor for penile erection. We sought to conduct a hemodynamic study in order to elucidate what extent of the actions of penile veins in penile erection, and possibly being an important contributor to impotence.

**Materials and Methods:** From March to August 2009, six male defrosted human cadavers were used for this study. Using colloid four sets of infusion cavernosometry were carried out with a flow rate of 35.0 ml/min while the intracavernosal pressure (ICP) was recorded before and after the deep dorsal vein (DDV), cavernosal veins (CVs) and para-arterial veins (PAVs) were removed respectively.

**Results:** The ICP can be reached up to 615 mmHg while a rigid erection was unexceptionally attained in all subjects, lasting significantly longer (p = 0.028), Vmax (p = 0.028), and pressure loss (p = 0.028). In cadaveric penises, after the removal of erection-related veins a rigid erection could be reached at the low flow rate of 5.5 – 8.0 ml/min colloid.

**Conclusion:** We, therefore, concluded that penile vein is categorically the determinant in penile erection since none of the current contributors of penile erection can be expressed in cadavers such as intracavernosinal, hormonal, arterial, and neurological, drugs effect, chronic systemic diseases, and psychogenic factors.

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**CABYR, A CANCER-TESTIS ANTIGEN EXPRESSED IN HUMAN LUNG CANCERS -- POTENTIAL BIOMARKER FOR LUNG CANCER**

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(Presented By: Panneerdoss Subbarayalu, PhD)

**Introduction and Objectives:** Calcium binding tyrosine phosphorylation regulated fibrous sheath protein (CABYR) is testis specific protein and normally expressed during postmeiotic stages of spermatogenesis. CABYR is a single copy gene, which undergoes alternative splicing involving two coding regions, coding region A (CR-A) and coding region B (CR-B) to encode six transcripts [isoform 1 through isoform 6].

**Methods:** RT-PCR analysis of 16 human lung squamous cell carcinomas and adenocarcinomas showed expression of isoforms 3 (88%), 5 (56%), 6 (56%), 2 (37%), and 1 (25%), but no evidence of isoform 4. 100% of lung squamous cell carcinomas [n=9] and 71% of adenocarcinomas [n=7] expressed isoform 3 mRNAs.

**Results:** Lung cancer cell lines NCI-H226 (squamous) and A549 (adenocarcinoma) showed expression of all six CABYR transcripts. At the protein level, all tumor specimens (n=9) showed expression of 50 KDA CABYR, 55% expressed 65 KDA CABYR (isoform 3); and 77% the 77 KDA (isoform 1). Tumor cell lines (n=10) expressed 77 KDAs (isoform 1), 65 KDAs (isoform 3), 50 KDAs and 25 KDAs (isoform 6) CABYR that matched bands in human spermatozoa. 2D SDS-PAGE analysis of CABYR in NCI-H226 cells revealed 25 immunoreactive spots with neutral pI (~6.5 - 7.5), consistent with predominant isoforms containing CR-B, while similar analysis of sperm showed predominantly acidic pI (~4.5-5.5). Immunoprecipitation of NCI-H226 cells showed 50 & 52 KDAs CABYR bands underwent tyrosine phosphorylation. Sera from infertile men with antisperm antibodies identifies CABYR as an autoantigen in 2D blots of sperm, as well as in immunoprecipitates from NCI-H226.

**Conclusion:** Together these studies indicate that CABYR, normally limited in expression to sperm, is highly expressed in human lung cancers and since it has the potential to be an autoantigen in humans, CABYR may have utility as a biomarker and therapeutic target in lung cancer.

**Funding:** This work was supported by NIH grant D43TW000654-14 from the Fogarty International Center and a grant from the Cancer Research Institute.

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**CAG AND GGN REPEAT LENGTHS AND CAG/GGN HAPlotYPES VARIATION IN ANDROGEN RECEPTOR GENE AND PROSTATE CANCER IN NIGERIA MEN**

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(Presented By: Oluyemi Akinloye, PhD)

**Introduction and Objectives:** Prostate cancer has become the number one cancer in Nigerian men and constitutes 11% of all male cancers. The growth of the prostate gland is dependent on circulating androgens and intracellular steroid signaling pathways. The effects of androgens are mediated through the androgen receptor (AR), a ligand-activated nuclear transcription factor encoded by AR gene. The common polymorphisms in Exon 1 of this gene; CAG and GGN repeats have been implicated as possible risk factor in prostate cancer. Thus far, existing supporting data are still scanty and none from sub-Saharan African population. Therefore, we have investigated the possible association between AR polymorphism repeats length (CAG and GGN) and prostate cancer in Nigerian men.

**Methods:** A total of 261 subjects consist of 70 prostate cancer patients, 68 benign prostate hyperplasia and 123 age matched apparently normal cases as controls. CAG and GGN repeats length were determined by fragment length analysis using GeneScan.

**Results:** The CAG repeats length of prostate cancer and benign prostate hyperplasia men compared to control were significantly different (p<0.05) with reduce length of CAG repeats showing a significant odd ratio in both cases. However, this significant pattern was not observed in GGN repeat which showed no significant different in percentage distribution between cases and controls (p>0.05). The different possible CAG and GGN haplotype variation shows no significant different between cases and controls (p>0.05), except that haplotype CAG<sub>21</sub> GGN<sub>21</sub> show a higher preponderant for controls.

**Conclusion:** This study, the first from sub-Saharan Africa supports the hypothesis that reduced CAG repeats length is a risk factor in prostate carcinoma and further extends similar possibilities to begin prostate hyperplasia.
Results: On average, we detected 524 ± 52.3 spots per gel. Forty-one spots were identified by MALDI-TOF/TOF, corresponding to twenty-eight different proteins. Combined, these spots represented 21.52% of the intensities of all spots. The most abundant proteins in the maps were low molecular weight spots identified as spermadhesins and BSP-like proteins. Two spermadhesin isoforms, bodhesins 1 (BDH1) and 2 (BDH2) and ram seminal vesicle proteins 14 (RSVP 14) and RSVP 22 represented 16.43% of the total intensity of all spots. Among the 41 identified spots, 35 were consistently present on all gels, representing matrix metalloproteinase 2, clusterin, 13-galactosidase and actin.

Conclusion: In conclusion, we identified different proteins in Santa Inês seminal plasma. According to their identity, they appear to be involved in different physiological events, such as sperm protection and capacitation. The study of the seminal plasma proteome of tropically-adapted rams will set the basis for understanding aspects of sperm reproduction associated with these genotypes.

ADVANCES IN UNDERSTANDING OF THE PENILE ANATOMY IN HUMAN BEINGS: A BRIEF OVERVIEW AND EVIDENCE OF MAMMALIAN EVOLUTION

Introduction: Has the human penile anatomy been well studied and the information sustainable? Could it provide a foundation for dictating medical strategies and evolution?

Methods: Dissecting, light, scanning and transmission electron microscopy and special stain were used for studies on the microstructures of human penis and representative quadrupeds. A brief overview was made.

Results: The tunica albuginea of the corpora cavernosa is a bi-layered structure with complete inner circular and incomplete outer longitudinal layers, which is absent between the 5 and 7 o'clock positions where two triangular ventral thickenings radiate from a bulbospongiosus form. On the dorsal aspect, there is the region called the dorsal thickening resulting from the ischiocavernous and continuing into the distal ligament, which acts as a spine within the glans penis. In the corpora cavernosa, skeletal muscle contains smooth muscle. This chamber meets the requirements for erection. In the corpus spongiosum, skeletal muscle partially entraps the smooth muscle in order to allow ejaculation when rigidly erect. It was elucidated that a deep dorsal vein, a couple of cavemosal veins, and two pairs of para-arterial veins are located between Buck's fascia and the tunica albuginea. An os penis is consistently noted in quadrupeds, in humans there is, however, an equivalent distal ligament with the same anatomical location and histology acts as a supporting trunk of the glans penis. This is evidence of evolution.

Conclusion: With this penile anatomical knowledge, a sustainable foundation can provide for daily medical questions, medical strategies, surgical solutions and evidence for species evolution.

SPERM FUNCTION / SEMEN ANALYSIS

IMPLICATION OF STORE-OPERATED CALCIUM CHANNELS ON P32 APPEARANCE AND SP32 ACTIVITY DURING THE ACROSOME REACTION IN BOAR SPERM

Christian Lessard, MSc1, Margaux Claverie1 and Janice L. Bailey, PhD1
1Laval University; 2IUT La Rochelle
(Presented By: Christian Lessard, MSc)

Results: On average, we detected 524 ± 52.3 spots per gel. Forty-one spots were identified by MALDI-TOF/TOF, corresponding to twenty-eight different proteins. Combined, these spots represented 21.52% of the intensities of all spots. The most abundant proteins in the maps were low molecular weight spots identified as spermadhesins and BSP-like proteins. Two spermadhesin isoforms, bodhesins 1 (BDH1) and 2 (BDH2) and ram seminal vesicle proteins 14 (RSVP 14) and RSVP 22 represented 16.43% of the total intensity of all spots. Among the 41 identified spots, 35 were consistently present on all gels, representing matrix metalloproteinase 2, clusterin, 13-galactosidase and actin.

Conclusion: In conclusion, we identified different proteins in Santa Inês seminal plasma. According to their identity, they appear to be involved in different physiological events, such as sperm protection and capacitation. The study of the seminal plasma proteome of tropically-adapted rams will set the basis for understanding aspects of sperm reproduction associated with these genotypes.

CAPACITATION INCREASES MITOCHONDRIAL MEMBRANE POTENTIAL IN BOAR SPERM

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(Presented By: Gianluca Paventi, PhD)

Introduction and Objectives: Capacitation is the ensemble of modifications, including membrane and intracellular signaling events; which occurs in vivo or in vitro, and is necessary for sperm fertilizing capacity. Our laboratory has previously shown that in porcine sperm, a Mr 32,000 tyrosine phosphorylated band, “p32”, appears in a calcium-dependent manner coincident with capacitation. The exact role and regulation of p32 appearance and sp32 activity are still not established, although it has been suggested that it is involved in the acrosome reaction. Indeed, sp32, a proacrosin binding protein, is a major component of the group of proteins that make up p32, and is tyrosine phosphorylated during capacitation. We hypothesized that p32 appearance and sp32 activity are coupled to the emptying of sperm calcium stores, subsequent store-operated calcium channel (SOCC) activity, and related to the acrosome reaction.

Methods: To test these hypotheses, fresh pig sperm were subjected to capacitation in the presence of modulators of sarcoplasmic and endoplasmic reticulum Ca²⁺-ATPases (SERCA), store-operated channel (SOC) and a calcium chelator.

Results: Thapsigargin, a SERCA inhibitor that should release calcium stores, markedly accelerated the appearance of p32, sp32 tyrosine phosphorylation and increased the % of spontaneous acrosome reactions (29±12% versus 7±4% for thapsigargin-free controls; p<0.05). p32 appearance, sp32 activity and the elevation of cytosolic calcium during capacitation were prevented by the inclusion of a calcium chelator, BAPTA-K+, in the medium (1±3% spontaneous acrosome reactions; p<0.05). Furthermore, thapsigargin could not override these effects of BAPTA-K+. The use of 2-aminooethyldiphenylboronate (2-APB), which inhibits both IP3-R and SOCC, and S&KF96365, which inhibits SOCC directly, reduced considerably p32 appearance, sp32 activity and spontaneous acrosome reactions when added with the thapsigargin.

Conclusion: These results suggest that IP3-R store-operated calcium entry regulates p32 appearance, sp32 activity and the onset of the acrosome reaction.

RESULTS: On average, we detected 524 ± 52.3 spots per gel. Forty-one spots were identified by MALDI-TOF/TOF, corresponding to twenty-eight different proteins. Combined, these spots represented 21.52% of the intensities of all spots. The most abundant proteins in the maps were low molecular weight spots identified as spermadhesins and BSP-like proteins. Two spermadhesin isoforms, bodhesins 1 (BDH1) and 2 (BDH2) and ram seminal vesicle proteins 14 (RSVP 14) and RSVP 22 represented 16.43% of the total intensity of all spots. Among the 41 identified spots, 35 were consistently present on all gels, representing matrix metalloproteinase 2, clusterin, 13-galactosidase and actin.

Conclusion: In conclusion, we identified different proteins in Santa Inês seminal plasma. According to their identity, they appear to be involved in different physiological events, such as sperm protection and capacitation. The study of the seminal plasma proteome of tropically-adapted rams will set the basis for understanding aspects of sperm reproduction associated with these genotypes.
Results: No difference between CM and NCM samples were found at 0h, thereby ruling out any effect due to the media. In contrast, at 3h, an increase in both the rate and extent of deltapsi generation was observed for CM DSC: the increase in deltapsi generation was about 70, 65, 45 and 40% for succinate, malate+glutamate, lactate and malate+pyruvate, respectively. Interestingly, no increase in deltapsi generation was found for ascorbate+TMPD+cyt c, thus ruling out a role for complex IV in the increase of deltapsi generation.

Conclusion: Together, these data show that during capacitation, the increased mitochondrial deltapsi is derived from increased respiratory chain activity with the exception of complex IV. This deltapsi increase could result from the enhanced progressive motility and protein phosphorylation associated with capacitation. The use of specific respiratory chain inhibitors will be useful to dissect the role of individual complexes in creating this increased deltapsi generation; furthermore use of mitochondrial uncouplers could ascertain whether the increased activity of mitochondrial respiratory chain is dispensable for capacitation.

THE ASSOCIATION OF AGING, OXIDATIVE STRESS AND DNA INTEGRITY IN HUMAN SPERMATOZOA

Edna Tirado, PhD1, Michele Marquette, PhD2, Joseph Musto, PhD2 and Benjamin Leader, MD, PhD3
1ReproSource Inc; 2UTMB

Introduction and Objectives: Oxidative stress (OS) is strongly correlated with adverse effects on cellular lipids, proteins and DNA, which result in defective sperm function and reduced fertility. The association of male age with OS, sperm DNA integrity and fertility has not been completely investigated. This study evaluated the association amongst age, oxidative stress, and sperm DNA integrity as measured by the oxidative stress adduct (OSA) test and the DNA fragmentation index (DFI) from sperm DNA fragmentation assay (SDFA).

Design: Comparative in vitro and retrospective studies.

Materials and Methods: Semen samples from 150 males from infertile couples (27-59 years old) were evaluated for OS and DNA integrity using the OSA and SDFA tests respectively. Data was grouped according to age, and the frequency of patients with abnormal OSA and/or DFI scores per age group was calculated. For comparative purposes, semen from 15 normozoospermic, young males (mean age ±SD: 27 ±2.5 years), were treated in vitro with hydrogen peroxide (200 μM H2O2). OSA and DFI scores were determined in both untreated and treated aliquots.

Results: A positive correlation was found between a) age and frequency with abnormal OSA scores (r=0.9938 p<0.0005); b) age and frequency with DFI scores (r=0.908 p< 0.0005); and c) frequency with both OSA and DFI abnormal scores (r=0.6958 p<0.0005). The treatment of semen from the 15 normozoospermic men with a mean age of 27 with hydrogen peroxide showed a significant increase in OSA and DFI scores between the control and treated groups. Treated samples demonstrated OSA and DFI scores similar to males from infertile couples above the age of 40: mean +/- SD OSA score 2.5 +/- 0.7 and 9.5 +/- 3.8 μM respectively, (t=3.21; p= 0.0077); and 2) mean +/- SD DFI score 6.3 +/- 1.0 and 36.8 +/- 3.9, (t=3.33 p<0.0062).

Conclusion: From semen from males of infertile couples showed that increasing age was associated with increased abnormalities in damage from oxidative stress, as determined by the OSA test, and abnormalities in DNA integrity as assessed by the SDFA test. In addition, comparative in vitro studies suggest that oxidative stress can change semen characteristics of young fertile men to resemble that of older, infertile men.

Funding: Financial Support: ReproSource Inc.

DNA FRAGMENTATION DETERMINED BY SPERM CHROMATIN DISPERSION TEST (HALOSPERM KIT) IS ASSOCIATED WITH DECREASED MOLTILITY AND AN ABNORMAL TOTAL MOTILE COUNT IN MEN OF INFERTILE COUPLES

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Introduction: Sperm DNA fragmentation index (DFI) is of growing significance in the evaluation of infertile couples; however, the most reliable and cost-effective laboratory test to measure DFI remains to be determined. The Halosperm Test Kit (Halo) measures DFI based on sperm chromatin dispersion. It only requires conventional bright-field microscopy and a turn-around time of 2 hours. This pilot study explores the utility of Halo in the evaluation of the male partner of infertile couples.

Objective: To assess the validity of Halo as a measure of DFI in a population of infertile couples seen at a single infertility center.

Methods: The de-identified data of semen analysis, total motile count (TMC), and DFI as measured by Halo of infertile couples (n=133) were reviewed retrospectively, and correlation analysis after log transformation was performed to look for associations between DFI and individual semen parameters. Standard semen analysis was performed according to the World Health Organization (WHO) 1999 4th Edition guidelines. Sperm decondensation was achieved according to the manufacturer's protocol by embedding sperm in a thin agar layer with subsequent denaturation, lysis, water rinsing, alcohol fixation, and staining using Stat III. TMC was used to subdivide semen analyses into Normal (≥20 million motile sperm; n=110) and Abnormal (<20 million motile sperm; n=23) sub-groups, and the mean DFI was compared using a Student t-Test.

SEMen BACTERIAL INFECTION IN HOLSTEIN BULLS INCREASES THE RATE OF Sperm DNA FRAGMENTATION BUT THIS DELETEROUS EFFECT CAN BE CONTROLLED USING QUINOLONES

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Introduction and Objectives: The dynamics of sperm DNA fragmentation (SDF) was assessed using commercial cryopreserved sperm samples from two cohorts of Holstein bulls. One cohort tested positive for the presence of bacterial infection (Bl) between 0 and 96 hours of incubation at 37°C after thawing (n=23), while the second cohort tested negative for the presence of Bl during a similar period of incubation time (n=24). Six straws from different ejaculates were randomly selected from each bull and examined for bacterial growth and SDF. In the cohort of straws of semen presenting Bl, contamination was positively detected immediately after thawing in 15% of the straws, whereas contamination was positively detected in half of the straws after 24 hours of incubation and in all straws after 96 hours of incubation. Microorganisms were identified by the polymerase chain reaction (PCR) sequencing method, examining amplified ribosomal RNA gene regions of the bacterial gene that codes for ARNr 16S, revealing the phyla: Bacteroidetes, Firmicutes, Proteobacteria, Cyanobacteria, Fusobacteria and Actinobacteria.

Results: When contaminated and non-contaminated groups were compared, differences in the frequency of sperm with fragmented DNA were not observed immediately after thawing, with an average of 3.65% ± 1.55% SDF. However, the rate of increase of DNA fragmentation (rSDF) with incubation time was higher in those sperm samples with the presence of Bl than those without. The estimated rate of SDF was on the order of 0.7% per hour in Bl samples, while being around 0.05% per hour in Bl-free samples. Moreover, whereas linear rates of SDF increase were the major trend in non-contaminated samples, logarithmic rates were predominant in contaminated samples. Similar experiments performed in semen samples treated with quinolones abolished the increase in SDF.

Conclusion: These results call attention to two main points: 1) sperm DNA fragmentation is directly related to semen bacterial infection and 2) sperm DNA fragmentation is greatly reduced after incubating sperm samples with quinolones.
Results: Group analysis identified a mean age, TMC and DFI of 36 years, 78.2 and 20.3% respectively. DFI was associated with the following semen parameters in decreasing significance: sperm motility ($r = -0.52$), sperm count ($r = -0.25$), and ejaculate volume ($r = -0.03$). The mean age in years for Normal and Abnormal was 36 and 39 respectively. The mean TMC for Normal and Abnormal was 93.3 and 6.3 respectively. The mean DFI was significantly different between Normal and Abnormal (p<0.001) and was 17.4 and 34.4 respectively (Figure 1).

Conclusions: DFI as measured by Halo is associated with decreased motility and an abnormal Total Motile Count. Future studies of fertility treatment outcomes will further define the utility of Halo in the evaluation of infertile couples.

Activity of the Na,K-ATPase α4 Isoform is Important for Motility and to Maintain Membrane Potential, Intracellular Ca2+ and pH in Rat Spermatzoa

Tamara Jimenez, Graduate Student, Gladis Sanchez, Research Associate, Eva Wertheimer, graduate student and Gustavo Blanco, MD, PhD

University of Kansas Medical Center (Presented By: Gustavo Blanco, MD, PhD)

Introduction and Objectives: The Na,K-ATPase is an ion transporter essential to numerous cell processes that depend on the transcellular gradients of Na+ and K+. The Na,K-ATPase consists of several isozymes, characterized by a particular α and β subunit composition. Among the α polypeptides, α4 is a sperm specific isoform that has characteristics that are highly unique and essential to sperm physiology. Here, we have explored the mechanisms by which α4 influences sperm function.

Methods: For this, we have taken advantage of the high sensitivity of α4 to ouabain. Using computer assisted sperm analysis; we have found that selective inhibition of α4 with ouabain affects rat sperm total motility and decreases different parameters of sperm movement, such as progressive displacement, beat cross frequency, and linearity.

Results: On the other hand, inhibition of α1, the other α isoform present in sperm, with higher doses of ouabain did not produce further changes in cell motility. In addition, ouabain inhibition of α4 increased intracellular Na+ and affected cell membrane potential, causing depolarization of the cells. Moreover, by interfering with α4 activity, ouabain increased calcium and diminished pH in sperm.

Conclusion: Altogether, these results suggest that the Na+ gradient generated by the α4 isomorph is important in controlling sperm Ca2+ and H+ in the cells. The relevance of these ions for sperm motility suggests that their control may be the mechanism by which α4 maintains motility and fertility of the male gametes.

Funding: Supported by NIH grants HD043044 and HD055763.

Direct Comparison of Two Processing Techniques on Sperm DNA Integrity

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Introduction and Objectives: The first goal of this study was to determine the effect that semen processing has on sperm DNA integrity. The second goal was to assess which processing technique (swim-up versus density gradient) results in a superior sample. DNA integrity was measured using a novel Toluidine Blue Assay. This was a side-by-side comparison in a hospital-based andrology laboratory.

Methods: Raw semen samples were collected from 32 patients and scored for routine semen analysis. Prior to discarding the specimens identical aliquots were divided and processed by density gradient centrifugation and sperm swim-up techniques. The Toluidine Blue Assay analyzed raw and processed samples. Stained slides were analyzed by light microscopy for dark, light, and medium stained cells spermatozoa.

Results: Both density gradient centrifugation and swim-up improved DNA quality compared to the unprocessed sample. However, the swim-up technique proved superior.

Conclusion: The swim-up technique generates a sperm sample with better chromatin integrity. Should chromatin integrity correlate with better pregnancy rates in IUI and IVF, respectively, the swim-up may be the sperm processing technique of choice for these procedures.

Comparison of Low and High Density Sperm Subpopulations in Bovine

Olivier D'Amours, MSc, Nancy Allard, Gilles Frenette, BSc, Marline Fortier, MSc, Pierre Leclerc, PhD and Robert Sullivan, PhD

Université Laval, Centre de recherche en biologie de la reproduction (Presented By: Oliver D'Amours, MSc)

Introduction and Objectives: Using the 2D DIGE technique, we previously compared detergent soluble protein fraction of cryopreserved sperm from bulls with high and low fertility scores. Eight proteins presented significant differences between the high and low fertility groups. The aim of the present study is to elucidate the apparent relationship between three of those proteins, i.e, ELSPBP1, BSP1, and PEBP1.

Methods: Using DIGE quantification, abundance of PEBP1 and BSP1 in sperm extract is highly but negatively correlated. Following sperm centrifugation on discontinuous Percoll gradient, ELSPBP1 and BSP1 are mostly found in the low-density subpopulation whereas PEBP1 is more abundant in the high-density subpopulation. Results: As assayed by flow cytometry, post-thaw viability of spermatozoa was highly but negatively correlated with the abundance of BSP1 in the sperm protein extract as quantified by DIGE. On the contrary, PEBP1 presented an inverse tendency. Interestingly, ELSPBP1 didn't show a significant relationship with post-thaw viability. ELSPBP1 and BSP1 are expressed in the epididymis and their presence on sperm is modulated during the epididymal transit. BSP1 is one of the major proteins of the seminal plasma and is acquired by spermatozoa at ejaculation. Once again, ELSPBP1 characterised the low-density subpopulation of cauda epididymal sperm but PEBP1 is equally distributed between the low and high density subpopulations.

Conclusion: ELSPBP1, PEBP1, and BSP1 presenting high affinity for phospholipids, differences in phospholipid content of membrane spermatozoa could exist between high and low density sperm subpopulations.

Funding: Supported by NSERC grant to RS and PL.

Comparison of Low and High Density Sperm Subpopulations in Bovine

Olivier D'Amours, MSc, Nancy Allard, Gilles Frenette, BSc, Marline Fortier, MSc, Pierre Leclerc, PhD and Robert Sullivan, PhD

Université Laval, Centre de recherche en biologie de la reproduction (Presented By: Oliver D'Amours, MSc)

Introduction and Objectives: The first goal of this study was to determine the effect on semen processing has on sperm DNA integrity. The second goal was to assess which processing technique (swim-up versus density gradient) results in a superior sample. DNA integrity was measured using a novel Toluidine Blue Assay. This was a side-by-side comparison in a hospital-based andrology laboratory.

Methods: Raw semen samples were collected from 32 patients and scored for routine semen analysis. Prior to discarding the specimens identical aliquots were divided and processed by density gradient centrifugation and sperm swim-up techniques. The Toluidine Blue Assay analyzed raw and processed samples. Stained slides were analyzed by light microscopy for dark, light, and medium stained cells spermatozoa.

Results: Both density gradient centrifugation and swim-up improved DNA quality compared to the unprocessed sample. However, the swim-up technique proved superior.

Conclusion: The swim-up technique generates a sperm sample with better chromatin integrity. Should chromatin integrity correlate with better pregnancy rates in IUI and IVF, respectively, the swim-up may be the sperm processing technique of choice for these procedures.

Funding: Supported by NIH grants HD043044 and HD055763.
CONCEIVEASE™, A NOVEL NON-SPERMICIDAL LUBRICANT, MAINTAINS SPERM MOTILITY AND CAN BE SAFELY USED FOR REPRODUCTIVE ISSUES

Kush Patel, MD, Sharon DeWitt, BS, Arthur Caire, MD, Mathew Coommen, MD, Anuj Sharma, Suresh Sikka, PhD and Wayne Helstrom, MD, FACS

(Presented By: Kush Patel, MD)

Introduction and Objectives: To evaluate the effects of a novel lubricant ConceiveEase™ (Sepal Reproductive Devices, Boston, MA) on sperm motility as compared to other lubricants.

Materials and Methods: Fresh human semen samples from 8 healthy volunteers (mean age 25.3 years) were collected and baseline motility evaluated within 1 hour of collection. Semen samples with >20% motility were centrifuged after addition of 5ml sperm wash media (SWM) to remove seminal plasma. Washed sperm pellet were resuspended in 1ml of SWM and equally divided into 4 groups. Sperm suspension was incubated with ConceiveEase™, Pre–Seed, mineral oil, and SWM (as control). Sperm motility (grades A+B+C) was evaluated as per WHO 4th guidelines, at time intervals of 1 minute, 15 minutes, 30 minutes, 120 minutes, and 240 minutes.

Results: Mean sperm motility for ConceiveEase™ did not decrease as compared to Pre–Seed or Mineral Oil over a period of 4 hours (shown in the control figure). In fact, ConceiveEase™ group maintained a close parity to the control figure. In fact, ConceiveEase™ group maintained a close parity to the control figure. Compared to Pre–Seed or Mineral Oil over a period of 4 hours (shown in the control figure). In fact, ConceiveEase™ group maintained a close parity to the control figure. Compared to Pre–Seed or Mineral Oil over a period of 4 hours (shown in the control figure).

Conclusion: ConceiveEase™ is an effective non-spermicidal lubricant as compared to other commercially available lubricants. Its advantages include use for semen collection in fertility centers, as a personal lubricant by couples, and for toxicological safety studies.

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MODIFICATION OF SPERM DNA FRAGMENTATION PARAMETERS AFTER XY SPERM SEX SORTING IN BOVINE

Michael E. Kjelland, PhD1, Miguel A. Ramirez, BS2, Carmen López-Fernández, PhD1, Francisco Crespo, PhD1, Kenneth M. Evans1, Juan F. Moreno, BS1 and Jaime Gosálvez, PhD2

(Presented By: Michael E. Kjelland, PhD)

Introduction and Objectives: Flow cytometric technology for the sorting of X/Y–chromosome-bearing sperm (sex selection) in mammalian species is currently used in research and commercial applications. An assessment of the level of Sperm DNA Fragmentation (SDF) produced after sperm sex selection in Bos taurus (n=20) was the goal of this study. The hypothesis was that sperm DNA damage could be reduced during the sex sorting process, due to a step during the sperm subpopulation separation methodology that removes nonviable and membrane compromised spermatozoa.

Methods: A dynamic assessment of SDF was made using the bull Sperm-Halomax® kit (Halotech DNA, Madrid, Spain) during an incubation time of 72 hours and a temperature of 37°C (Figure 1). MoFlo SX XDP™ (Beckman Coulter, Miami FL) sperm sorters were used for sperm sex selection. Results showed that the level of SDF is reduced in both X and Y sex sorted samples, 63% on average, while the level of SDF increases within the dead sperm subpopulation. However, when conventional and sex sorted samples are compared, the sperm DNA longevity is lower in sexed sperm, with a detectable window for sperm DNA damage occurring between 24 and 48 hours of sperm incubation. The efficiency in decreasing the SDF after XY sex selection varied for individual bulls (P< 0.05). Furthermore, in sex sorted sperm samples, the baseline level of SDF did not account for the dynamic behaviour of DNA longevity as each individual bull presented a different rate of increase in the level of SDF.

Results: Cytofluorimetry allows for the separation of a large portion of DNA damaged sperm cells, which are mainly found in the X/Y sex sorted non-viable or membrane compromised sub-population. The baseline level of SDF is higher in conventional than in X/Y sex sorted samples, but the overall rate of SDF is higher in X/Y sex sorted samples than in conventional samples. Future research for improving X and Y sex sorting should be conducted to identify and then minimize the damage for each step of the sorting process.

Funding: This research was funded by Sexing Technologies.

Figure 1. Bos taurus sperm after four hours of incubation at 37°C using the SCD procedure for detecting DNA fragmentation (red arrows): a) sex sorted and b) conventional.

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SIMULTANEOUS ANALYSIS OF DNA FRAGMENTATION AND 8-OXOGUANINE IN HUMAN SPERM

Rebeca Santiso, PhD1, María Tamayo, PhD1, Jaime Gosálvez, PhD1, Marcos Meseguer, PhD2, Nicolás Garrido, PhD2 and José Luis Fernández, MD, PhD1

1 INIBIC – Complejo Hospitalario Universitario A Coruña, Unidad de Genética; 2 Unidad de Patología, Universidad Autónoma de Madrid, Spain; 3 UVI Valencia, Universidad de Valencia, Spain

(Presented By: Rebeca Santiso, PhD)

Introduction: After the Sperm Chromatin Dispersion (SCD) test, nuclei with DNA fragmentation show very small or no halos of DNA dispersion, whereas those sperm without DNA fragmentation reveal large halos. Sequential application of a fluorescent 8-oxoguanine (8-oxoG) probe allowed the simultaneous determination of DNA fragmentation and oxidative damage on the same sperm cell.

Objectives and Methods: 1) Assay validation by incubation of a sperm sample with hydrogen peroxide (H2O2), which decomposes into hydroxyl radicals producing mainly 8-oxoG, sodium nitroprusside (SNP), a nitric oxide donor that produces 8-nitroguanine, and DNAasel that only produces DNA breakage. 2) Study of the DNA fragmentation level and 8-oxoG labeling in 31 human sperm samples. 3) Comparison of 8-oxoG labeling in patients with 30.9–59.0% of sperm cells with fragmented DNA, nine that gave pregnancy and eleven that did not after ICSI.

Results: H2O2 induced DNA fragmentation and 8-oxoG in the same sperm cell. SNP also resulted in DNA fragmentation, with a very discrete increase in 8-oxoG labeling. Finally, DNAasel incubation produced DNA fragmentation without 8-oxoG labeling. This later result indicates that sperm DNA fragmentation may potentially be independent of oxidative DNA damage. Nevertheless, in all sperm samples examined, increased levels of 8-oxoG were present practically only in those spermatozoa with fragmented DNA, even in samples that did not get a pregnancy.

Conclusion: Coincidence of DNA fragmentation and 8-oxoG within the same sperm cell suggests a link between both damage types, initially discarding this kind of extensive oxidative damage outside the population with fragmented DNA.
Differential Modifications of Human Sperm Peroxiredoxin 6 by Reactive Oxygen Species

Stefan Patrascu, BSc and Cristian O'Flaherty, DVM, PhD
Urology Research Laboratory, Surgery Dept., McGill University-MUHC
(Presented By: Cristian O'Flaherty, DVM, PhD)

Introduction and Objectives: Excessive levels of reactive oxygen species (ROS) in spermatozoa are associated with infertility. The increase in ROS levels, or oxidative stress, is the result of an excessive production of ROS and/or a decrease in the antioxidant defense system, causing serious cell injury and even cell death. Several malignancies such as varicocele, testicular torsion and idiopathic infertility have an oxidative stress as an important component of their pathogenesis. Peroxiredoxin 6 (PRDX 6) is part of a newly discovered antioxidant enzyme family involved in the protection of somatic cells against oxidative stress. In such conditions, the catalytic cysteine at the active site of PRDX 6 is oxidized, which inactivates the enzyme. Further oxidation will promote the production of the sulfonated form (PRDX 6-S02). We previously demonstrated the presence of PRDX 6-S02 antibodies.

Methods: Spermatozoa from healthy volunteers were incubated with increasing concentrations of hydrogen peroxide (H2O2), tert-butyl hydroperoxide (tert-BHP) or peroxynitrite (ONOO-) for 30 minutes at 37°C. Then, sperm samples were immunoblotted with anti-PRDX 6 and anti-PRDX 6-S02 antibodies.

Results: We observed that under non-reducing conditions, the PRDX 6 doublet bands seen at 24/26 kDa in untreated samples became a single strong 26 kDa band at all H2O2 and tert-BHP concentrations, and at the highest concentration of ONOO-. Only high levels of H2O2 promoted formation of higher molecular mass complexes. Furthermore, H2O2 and tert-BHP were found to decrease the amount of PRDX 6 in the cytosolic and Triton-soluble fractions. As for PRDX 6-S02, its amount was only increased following incubation with the highest concentration (2 mM) of H2O2 or tert-BHP.

Conclusion: In conclusion, the reactivity of PRDX 6 and PRDX 6-S02 to reactive oxygen species (ROS) in vitro suggests that this enzyme plays a role as a regulator of ROS action and as an antioxidant in human spermatozoa.

Funding: Funded by CIHR.

Sperm Protection by Milk: Binding of Boar Binder of Sperm1 (BSP1) to Milk Proteins

Marie-France Lusignan, MSc and Puttaaswamy Manjunath, PhD
University of Montreal
(Presented By: Marie-France Lusignan, MSc)

Introduction and Objectives: Seminal plasma of mammals contains a family of proteins called Binder of Sperm (BSP), which are secreted by seminal vesicles. Our extensive studies in bovine have shown that these proteins bind to sperm at ejaculation and induce a continuous cholesteryl and phospholipid removal from the sperm membrane that is deleterious to sperm storage. Interestingly, low-density lipoproteins (LDL) from egg yolk, a constituent commonly used in extenders for sperm preservation, bind BSP proteins. This interaction is believed to shield sperm against the detrimental effect of BSP proteins. Heated skim milk is another constituent widely used to preserve mammalian sperm. We recently demonstrated that bovine BSP proteins could bind to milk proteins and this interaction would protect sperm in a similar way as egg yolk do. Boar seminal plasma contains one protein homologous to BSP proteins, boar BSP1 (previously called pB1). In the present study, we provide evidence that boar BSP1 share similar binding properties as the bovine BSP proteins by binding to milk proteins.

Methods: Heated skim milk was fractionated on a Sepharose CL 4B column. The isolated fractions were then incubated with purified boar BSP1 protein and rechromatographed on the same column. The proteins in various fractions were subjected to SDS-PAGE, transferred to PVDF membranes and probed with antibodies against boar BSP1 protein.

Results: Heated skim milk was separated into three fractions (F1, F2 and F3). F1 contained high molecular weight complexes composed of whey proteins (alpha-lactalbumin and beta-lactoglobulin) and kappa-casein. F2 contained all caseins and F3 contained small peptides, salts and sugars. Co-incubation of F1 or F2 with boar BSP1 followed by gel filtration and immunoblot analysis of eluted fractions indicated that boar BSP1 co-eluted with F1 but not with F2.

Conclusion: Heated skim milk was separated into three fractions (F1, F2 and F3). F1 contained high molecular weight complexes composed of whey proteins (alpha-lactalbumin and beta-lactoglobulin) and kappa-casein. F2 contained all caseins and F3 contained small peptides, salts and sugars. Co-incubation of F1 or F2 with boar BSP1 followed by gel filtration and immunoblot analysis of eluted fractions indicated that boar BSP1 co-eluted with F1 but not with F2.

Funding: Supported by NSERC, FQRNT and FESP, UdeM.

Motile Human Sperms in a Microfluid Device—A Novel Treadmill for Counting and Sorting Sperms

Vincent F.S. Tsai, MD, MBmEng1, Ju-Ton Hsieh, MD, MD, Hong-Chiang Chang, MD, Andrew M. Wu, PhD1, Yo-An Chen, Master1, Zi-Wei Huang, Master1 and Fu-Shan Jaw, Ph D1
1Tenchen Medical Group, Institute Biomedical Engineering of National Taiwan University; 2National Taiwan University Hospital; 3Institute of Applied Mechanics, National Taiwan University; 4Institute of Biomedical Engineering, National Taiwan University
(Presented By: Vincent F.S. Tsai, MD, MBmEng)

Introduction and Objectives: As male infertility becomes an epidemical problem, and non-home used tests are bothersome and embarrassing, an easier and objective means for semen analysis is needed. This study presents a novel microfluid design for motile human sperm counting and sorting.

Methods: Human semen collected from clinical-indicated patients at urological OPD of NTUH was tested by this microfluid design, microscope and Sperm Quality Analyzer (SQA llB). Motile sperm counts by an impedance sensor in this microfluid design were compared with the results of microscope and SQA. Motile sperms were instilled into this design for sorting according to different swimming velocity.

Results: There was good correlation for motile sperm count results between this design and microscope and SQA. Motile sperms were also sorted into 3 classes of different swimming velocity.

Conclusion: The design of motile human sperm in microfluid could measure motile sperm count and sort motile sperm of different swimming velocity. The technology of microfluidics could be utilized for home used sperm counting and sorting in sperm banks.
SMALL VARIATIONS IN CRUCIAL STEPS OF TUNEL ASSAY COUPLED TO FLOW CYTOMETRY GREATLY AFFECT MEASURES OF SPERM DNA FRAGMENTATION.

Monica Muratori, PhD,1 Lara Tamburrino, PhD, student1, Antonietta Costantini, PhD2, Sara Marchiani, PhD2, Claudia Giachini, PhD, student1, Ilaria Laface, PhD, student2, Maria C. Meriggiola, MD,2 Gianni Forti, MD2 and Elisabetta Baldi, PhD2

1Dept. of Clinical Physiopathology, Sexual Medicine and Andrology Unit, University of Florence; 2Department of Obstetrics and Gynecology, Center for Reproductive Health, University of Bologna

(Presented By: Monica Muratori, PhD)

Introduction and Objectives: Techniques assessing sperm DNA damage are numerous and heterogeneous. There are two main types of assays: direct and indirect ones. The former directly detect the amount of sperm DNA damage whereas the latter reveal the effect of an exogenous insult on sperm chromatin. In addition, even considering the same type of technique, different strategies to reveal and/or quantify sperm DNA damage are used. Finally, these techniques, except SCSA (Sperm Chromatin Structure Assay), lack standardized protocols to which adhere to minimize inter-laboratories variations.

Methods: In this study we investigated the effects of some of the many variants by which TUNEL assay is performed on the measures of sperm DNA fragmentation by flow cytometry. In addition, by using an established procedure, we determined the precision of the technique by calculating intra-assay coefficients of variation (CVs).

Results: We found that the concentration of the fixative, the time of storage of fixed samples, the fluorochrome used to label DNA breaks and the method to analyze flow cytometric data, all greatly affect the measures of sperm DNA fragmentation. In particular, after 1-week storage in paraformaldehyde (PFM), the coefficient of variation was 28.3±17.9% (mean±SD, n=9 determinations). We found that the treatment with paraformaldehyde produces an additional damage in most of samples, as revealed by both flow cytometric and blot analysis of tunnel-labelled sperm DNA, suggesting that also TUNEL can be considered an indirect assay when performed in semen samples treated with such fixative reagent. We also found that many methods to analyse data yield results that, albeit correlating, are different and differently associated to semen quality.

Conclusion: Finally, TUNEL assay shows low intra-assay CVs (3.7±2.4%, mean±SD, n=8), resulting a quite precise technique when performed in established conditions.

SUMO-1YLATION OF HUMAN SPERMATOZOA AND ITS RELATION WITH SEMEN QUALITY

Sara Marchiani, PhD1, Lara Tamburrino, PhD, student1, Lucia Galiano, student in Biotechnology1, Danièle Nosi, researcher2, Valentina Sarli, student in Biotechnology1, Gianni Forti, MD, full professor in Endocrinology2, Elisabetta Baldi, associate professor1 and Monica Muratori, researcher1

1Dept. of Clinical Physiopathology, Sexual Medicine and Andrology Unit, University of Florence; 2Dept. of Anatomy and Histology, University of Florence

(Presented By: Sara Marchiani, PhD)

Introduction and Objectives: SUMOylation is a post-translational protein modification involved in the regulation of essential cell functions. Of interest, recent data indicate that SUMOylation of mitochondrial proteins is associated with loss of morphology and function of these organelles.

Methods: In view of the essential role played by mitochondria in sperm functions, we investigated the expression of SUMOylated proteins in human ejaculated spermatozoa by means of western blot, immunofluorescence and cyttofluorometric analysis.

Results: We found several Sumo-1 and Sumo-2/3ylated proteins in PureSperm selected spermatozoa in a molecular weight range of 25–85 kDa. By immunofluorescence and immunofluorometric fluorescence analysis, we demonstrated that SUMOylated proteins are mainly located in the nucleus and in the midpiece. In particular, co-localization between Sumo-1 and mitotracker (a specific mitochondrial probe) fluorescence signals indicates the occurrence of Sumo-1ylated proteins within mitochondria. By cyttofluorometric analysis, we investigated the percentage of SUMO-1ylated spermatozoa in 52 consecutive male partners of infertile couples undergoing semen analysis in our Laboratory and correlated the obtained values with semen parameters.

Conclusion: We found that the percentage of SUMO-1ylated spermatozoa was inversely correlated with total (r=0.3, p<0.02) and progressive motility (r=-0.27, p<0.05), suggesting an involvement of SUMOylation in the regulation of this sperm function.

LIGAND BINDING PROPERTIES OF A RECOMBINANT MURINE BINDER OF SPERM HOMOLOG (BSPH1)

Genevièvre Plante and Puttasawmy Manjunath, PhD
Maisonneuve-Rosemont Hospital Research Centre
(Presented By: Geneviève Plante)

Introduction and Objectives: Sperm capacitation is a maturation step that is essential in order for the spermatozoa to be able to fertilize an oocyte. A family of proteins, the Binder of Sperm (BSP), are known to bind choline phospholipids on the sperm membrane and thus promote capacitation in species such as bovine and porcine. These BSP proteins, secreted by the seminal vesicles, share similar characteristics such as binding to gelatine, heparin, glycosaminoglycan (GAG) and to lipoproteins (HDL, LDL). Recently, BSP-homologous genes have been identified in the epididymis of human (BSPH1) and mice (Bsp1h and Bsp2h). The objective of the current study was to determine if Bsph1 shares some binding characteristics with the other BSP family proteins and therefore could play a similar role in sperm function.

Methods: Because a very small amount of BSP homologs are found in human and mouse, we produced recombinant Bsph1. Since BSP proteins contain 4 disulfide bridges, we used Origami (DE3) cells, which contain a pET32a vector, which adds a thioredoxin tag to the expressed proteins by affinity chromatography and protein electrophoresis on agarose gels or by SDS-PAGE followed by immunoblot analysis.

Results: The preliminary data suggests that Bsph1 binding weakly to HDL, LDL, gelatine and heparin. These results demonstrate that the recombinant protein produced seems to have correctly folded and that it shares some characteristics with other BSP family proteins.

Conclusion: In future studies, the confirmation of the implication of Bsph1 in sperm function will identify a new factor with an impact on murine fertility. Since human (BSPH1) and mouse (Bsp1h) BSP homologs are orthologous proteins, these results could also be true for the human BSP-homolog.

Funding: Supported by NSERC, CIHR and FESP of university of Montréal.

THE TEMPERATURE OF CENTRIFUGATION IS IMPORTANT DURING SPERM INTRA-UTERINE INSEMINATION (IUI) PREPARATION I. KINEMATICAL

Elizabeth Elefano, Manuel Lomas, David Pitts, Rossana Cromwell, George Grunert and Wan-Song Wun
OGA
(Presented By: Elizabeth Elefano)
**Introduction and Objectives:** Studies show that using two-layer Percoll separation significantly improves semen characteristics for intra-uterine insemination (IUI). Due to instrumental limitation, traditionally IUI centrifugation is performed at room temperature. The impact of sudden temperature change, i.e. from incubator at 37 degrees C to centrifuge at room temperature, is unknown. With the recent availability of centrifuges that can maintain temperature at 37 degrees C, this study examines the potential temperature shock on the sperm characteristics.

**Materials and Methods:** The study was approved from exemption from Institutional Review Board. Eighteen discarded semen samples were used and each sample was split into two equal parts. Each part was processed identically except the temperature during centrifugation. Becton Dickinson Dynac II centrifuge was used for room temperature processing while the Eppendorf 5702 RH centrifuge was used for the 37 C processing. Both centrifuges performed at 1500 rpm with 400 g centrifugal force. The Spermatozoa kinematical parameters were then measured by Hamilton Thorne CASA system. The motility (MOT), rapid motility (R-MOT), curve linear velocity (VCL), amplitude of lateral head displacement (ALH), tail beat frequency (BCF), linearity (LIN), and hyperactivation (HA) were examined at 0, 1, 2 hour after processing. Least Square means and P-values are based on repeated measures mixed model analysis including temperature and time as the repeated factors.

**Results:** Sperm velocities (Shibahara et al, 2004), anterior ALH (Hirano et al, 2001), and HA (Liu et al, 2007) have been correlated with the capability of fertilization. In this study, the ALH and HA parameters were significantly improved by processing at 37 degrees C versus 25 degrees C.

**Conclusion:** This observation suggests centrifugation at 37 degrees C will improve IUI pregnancy rate. It waits for further clinical study to confirm the indication.

**Table 1** The effect of 8-Br-cAMP on the sperm kinematical characteristics

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<th>MOT</th>
<th>R-MOT</th>
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**Table 2** Functional challenger temperature on sperm membrane integrity

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<th>Slashed analysis</th>
<th>Apoptotic index</th>
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THE TEMPERATURE OF CENTRIFUGATION IS IMPORTANT DURING SPERM INTRA-UTERINE INSEMINATION (IUI) PREPARATION. II. FUNCTIONAL EXAMINATIONS

Elizabeth Elefaro, Manuel Lomas, David Pitsa, Cherie Morgan, Armando Mejia and Wan-Song Wun

OGA

(Submitted by: Elizabeth Elefaro)

**Introduction and Objectives:** By utilizing density gradient centrifugation at 37 degrees C, the processed spermatozoa has significantly better sperm kinematical characteristics (R-MOT, ALH, LIN, and HA) after 2 hours incubation as compared to centrifugation at room temperature (25 degrees C). This study is to examine the differences of functional capabilities of spermatozoa between control and experimental group. The design includes challenging the spermatozoa with 8-Bromo-cAMP (8-Br-cAMP), examining the initial apoptotic phenomenon of externalization of cell membrane phosphatidylserine, and hypo-osmotic swelling test (HOS).

**Materials and Methods:** The study obtained exempt status from IRB. Eighteen available semen samples were processed as Study I. The spermatozoa kinematical parameters are then measured by Hamilton Thorne CASA system. After two hours post IUI preparation, the spermatozoa were challenged with 0.5 mM 8-Br-cAMP. The kinematical parameters were taken at half hour and 1-hour point. The Annexin V kit (PromoKine inc, Heidelberg, Germany) to detect initial apoptotic phenomenon (Apoptotic index). The HOS followed the protocol of Jeyendran et al (1984). Least Square means and P-values are based on repeated measures mixed model analysis including duration of challenging by 8-Br-cAMP, centrifugation temperature, and time as the repeated factors.

**Results:** Functional challenging is a way to examine the healthy condition of spermatozoa. The 8-Br-cAMP challenging shows a significant enhancing of sperm kinematical characteristics (VCL, ALH, BCF, LIN, HA). This phenomenon and the decrease of sperm kinematical characteristics after preparation (from Study I) show the capability of spermatozoa significantly compromised with room temperature processing. The results of apoptotic index and HOS also show the spermatozoa losing membrane integrity by traditional room temperature centrifugation.

**Conclusion:** By utilizing density gradient centrifugation at 37 degrees C, the processed spermatozoa has significantly better sperm kinematical characteristics (R-MOT, ALH, LIN, and HA) after 2 hours incubation as compared to centrifugation at room temperature (25 degrees C). This study is to examine the differences of functional capabilities of spermatozoa between control and experimental group. The design includes challenging the spermatozoa with 8-Bromo-cAMP (8-Br-cAMP), examining the initial apoptotic phenomenon of externalization of cell membrane phosphatidylserine, and hypo-osmotic swelling test (HOS).

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GENES INVOLVED IN REPRODUCTIVE PROCESSES (RP) AMONG THOSE GENES DIFFERENTIALLY EXPRESSED (GDE) FROM INFERTILE MALES' (IM) SPERM SAMPLES (SS) UNDERGOING INTRA CYTOPLASMIC SPERM INJECTION (ICSI) ACHIEVING OR NOT PREGNANCY

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(Presented By: Sandra Garcia-Herrero)

**Introduction:** Basic SS analysis has limited predictive power to forecast pregnancy achievement in ICSI in some measure because the lack of information regarding the molecular causes of these failure attempts related with sperm.

**Objective and design:** Work's aim is to use microarray technology to characterize differential expression profile (EP) between SS achieving (group P) or not pregnancy (group NP) in ICSI cycles, and to compare our results with the current knowledge of genes already described to be implicated in RP. Nested cases and controls study. We choose 5 cases where an oocyte donor donates oocytes to two couples undergoing ICSI cycle, one get pregnant and second doesn't achieve pregnancy. Finally 10 SS (5 that achieved pregnant (group P) vs. 5 don't achieve (group NP) were obtained from IP undergoing ICSI cycle with oocytes from young donors. After freezing aliquots of the SS employed for ICSI treatment their respective mRNA EP were compared.

**Material and Methods:** Sperm (Sp) mRNA was extracted using Trizol protocol. RNAs were analyzed on Agilent Bioanalyzer 2100. GDE at least twice P and NP SS, were systematically searched within the specific Gene Ontology (GO) terms list related to RP: acrosome formation and reaction, binding of Sp to zona pellucida, copulation, embryo implantation and development, female pregnancy, fertilization, fusion of Sp to egg plasma membrane, male gamete and gamete generation, genitalia, germ cell, gonad placental and spermatid development, insemination, matting, reproduction, Sp competition and motility, Sp chromatin condensation, Sp egg recognition and spermogenesis. GO terms describe gene products depending of their associated biological processes in different steps (www.geneontology.org).

**Results:** 44 GDE were found in group P and 5 GDE in NP. Among them, we found 2 genes involved in some of these processes in group P: SPP1 (related with embryo implantation, female pregnancy and reproduction) and CXC4R (related with reproduction). Only 1 gene was found to be already associated to RP in group NP: ANKR07D (associated with male gonad development and reproduction).

**Conclusions:** This work reveals differences between EP from SS that achieved pregnancy vs. those unable and the limited information about RP. These differences could be potentially employed to detect ICSI success markers; nevertheless further knowledge of the molecular basis of Sp physiology is needed in order to improve diagnostic efficiency of Sp analysis.
THE INTAKE OF A SYMPATHOMIMETIC DRUG COMPENSATES FOR THE SEMINAL EMISSION DYSFUNCTION AND SEVERE OLGIZOOOSPERMIA CAUSED BY THE USE OF A SNRI ANTIDEPRESSANT PRESCRIBED FOR GENERALIZED ANXIETY DISORDER/PERIPHERAL NEUROPATHIC PAIN
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Introduction and Objectives: Semen emission is a concerted event under control of the central nervous system (i.e., sympathetic nervous system). Ejaculatory dysfunction can be experienced by men taking antidepressants belonging to the class of selective serotonin reuptake inhibitors (SSRIs) and/or serotonin-norepinephrine reuptake inhibitors (SNRIs). Ejaculatory dysfunction caused by the intake of SSRIs/SNRIs (dose dependent effect) results mainly in delayed ejaculation, severe oligozoospermia and/or virtual azoospermia. On the other hand, sympathomimetic drugs have been used to stimulate epididymal contractions due to aperistalsis, and also to reestablish antegrade ejaculation for cases involving retrograde ejaculation. In this study, we present a case in which a severe oligospermic patient taking a SNRI antidepressant was concurrently treated with a sympathomimetic drug to stimulate seminal emission and improve the sperm count.

Methods: A 28-year-old man presented to our clinic a main complaint of infertility caused by severe oligozoospermia/ virtual azoospermia. The patient had been prescribed an SNRI antidepressant for generalized anxiety disorder/peripheral neuropathic pain (Duloxetine; 60 mg/d). A trial with a sympathomimetic drug (Pseudoephedrine) was commenced to determine if stimulation of the epididymal contractions could be achieved with a subsequent improvement of the sperm output. Intake of pseudoephedrine (60 mg) was initiated on the day before the semen analysis and the last dose was taken 1 hour before semen collection. The patient was monitored for a period 8 weeks, during which semen analyses were performed at 2-week intervals. In addition, pseudoephedrine was intermittently administered during this period to determine a cause-effect relationship was taking place.

Results: The semen concentration, count and normal morphology were significantly improved after intake of the SNRI/pseudoephedrine as compared to semen specimens produced when taking the SNRI alone. The total sperm count was above normal values in two of the semen analyses following intake of pseudoephedrine.

Conclusion: Intake of pseudoephedrine as applied in this study neutralized the negative impact caused by the systemic effect of the SNRI, which resulted in severe oligozoospermia/virtual azoospermia. A cause-effect relationship was established for the role of the inhibitory effect of the SNRI, and the stimulatory effect of pseudoephedrine in regards to sperm emission.
METABOLIC PATHWAYS (MP) AFFECTED AMONG GENES DIFFERENTIALLY EXPRESSED (GDE) FROM INFERTILE MALE'S (IM) SPERM SAMPLES (SS) UNDERGOING INTRAUTERINE IMSEMINATION (IUI) ACHIEVING OR NOT PREGNANCY.

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(Presented By: Marcos Meseguer, PhD)

Introduction: Male fertility evaluation is usually focused on basic SS analysis, remaining molecular factors as mRNA presence within ejaculated spermatozoa. Our group defined a list of GDE from IM achieving pregnancy (group P 756 GDE) compared with those who didn't (group NP 194 GDE) by microarray technology.

Objective and Design: Study's aim was to analyze by means of bioinformatic tools these lists searching for MP affected in both groups. Nested cases and controls study with 20 SS (group P, n=10 and group NP, n=10) obtained from IM undergoing their first homologous IUI cycle presenting normal sperm count and motility (WHO criteria) parameters, having their partner's normal infertility work-up investigation results.

Material and Methods: SS mRNA was extracted using Trizol protocol, suspended in DEPC-treated water and frozen at -80°C until the microarray experiments were performed. RNAs were analyzed on Agilent Bioanalyzer 2100. The results were evaluated with the DAVID Gene bioinformatics tool (http://david.abcc.ncifcrf.gov/).

Results: 12 MP were statistically affected (SA) in group P; Adhesion and Diapedesis of Granulocytes, B cell receptor signaling pathway (SP), Toll-like receptor SP, NK cell mediated cytotoxicity, Epithelial cell signaling in H. pylori infection, Jak-STAT SP, Hematopoietic cell lineage, Chondroitin sulfate biosynthesis, Antigen processing and presentation, Leukocyte transendothelial migration, Integrins (these molecules mediate interaction sperm-egg) and Cytokine-cytokine receptor (participate in reproductive events such as implantation and placentation interaction). 4 MP were SA in group NP: Urea cycle and metabolism of amino groups, Glycerolipid, Arachidonic acid (from prostaglandins are synthesized) and Tryptophan metabolism.

Conclusion: The differences in the expression profiles between SS which achieved pregnancy vs. those unable in assisted reproduction also affect complex systems such as MP some of them already related with sperm fertilization process.

ANDROGENS / ENDOCRINOLOGY

ABNORMAL PATTERNS OF INHIBIN B AND ANDROGEN RECEPTOR IMMUNOEXPRESSION IN SERTOLI CELLS OF SENESCENT MEN AND CHRYSTORCHID PATIENTS

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(Presented By: Javier Regadera, MD, PhD)

Introduction and Objectives: The presence of Androgen Receptor (AR) and serum Inhibin-B (I-B) has recently been correlated to Sertoli cell function in normal and infertile men; however, conflicting data have been reported in subfertile patients. The present study was undertaken to explore: 1) the relationship between immunohistochemical (IH) expression of I-B and AR and the stages of the seminiferous epithelium; 2) the alterations in immunoeexpression of I-B and AR present in testicular atrophy of elderly men and in dysgenetic Sertoli cells found in pubertal chryptorchid testes.

METHODS: IH determinations of I-B and AR were carried out in normal and hCG-seminiferous tube of infertile testicular biopsies obtained from elderly men with prostate cancer and in 17 postpubertal testes of young patients with undescended testes, using Dako anti-I-B (1/400 dilution), and anti-AR (1/100 dilution) antibodies. Quantification of I-B expression in basal and adluminal Sertoli cell cytoplasm was performed with a Leica software in a 3000 m2 area. The positivity index of AR in Sertoli cell nuclei was also obtained.

RESULTS: In normal seminiferous tubules, I-B expression was significantly higher in the basal than in the adluminal Sertoli cell cytoplasm. In relation to stages of spermatogenesis, a low expression of I-B in the adluminal pole of Sertoli cells was observed in the areas next to both immature (p < 0.001) and mature spermatids associations (p < 0.001). In tubules with focal hippospermatogenesis, I-B expression was mildly decreased but in tubular atrophy, the decrease was very pronounced in Sertoli cells adjacent to areas with absence of primary meiotic spermatocytes. (p<0.001). This change correlated with negative AR expression in Sertoli cell nuclei. In postpubertal chryptorchidism, most dysgenetic Sertoli cells evidenced low or absent I-B expression and minimal or negative AR reactivity in its round or elongated nuclei.

Conclusion: The present data shows a strong association between Sertoli cell functional impairment and spermatogenic deficiency. The Sertoli/ spermatogenic deterioration in chryptorchidism is probably due to the existence of a primary testicular damage probably originated during fetal differentiation.

EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON DEPRESSIVE SYMPTOMS, LOW VITALITY AND SEXUAL DYSFUNCTION IN HYPOGONADAL MEN WITH THE METABOLIC SYNDROME: THE MOSCOW STUDY

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(Presented By: Farid Saad, DVM, PhD)

Introduction and Objectives: Low testosterone levels in men are associated with the metabolic syndrome as well as with depressive symptoms, low vitality and sexual dysfunction. This study tested the effects of testosterone administration on the above subjective symptoms in hypogonadal men with the metabolic syndrome (MetS).

Methods: In a randomized, placebo-controlled, double-blind, phase III trial (ClinicalTrials.gov identifier: NCT00696748), 184 men suffering from both the MetS and hypogonadism were included. They were treated for 30 weeks with either parenteral testosterone undecanoate (TU: 1000 mg) testosterone undecanoate, at baseline, and after 8 and 18 weeks; Nebido®; Bayer Schering Pharma, Berlin, Germany) or placebo injections. 105 (92.9%) men receiving TU and 65 (91.5%) receiving placebo completed the 30-week trial. The Beck Depression Inventory (BDI), Aging Males' Symptoms (AMS) scale, and International Index of Erectile Function 5-item (IIEF-5) scale at baseline, 18 and 30 weeks were analysed using multilevel analysis.

Results: The 184 men were aged mean 52.1 years old (SD 9.6; range 35–69), with a mean body mass index of 35.5 kg/m2 (SD 6.7; range 25.1–54.8), and a mean total testosterone level of 8.0 nmol/L (SD 4.0). There were significant improvements in BDI (mean difference vs. placebo after 30 weeks: -2.5 points; 95% confidence interval [CI] -0.9; -4.1; P=0.003), AMS (-7.4 points; 95% CI: -10.5; 1.9; P=0.001), and IIEF-5 +3.1 points; 95% CI: +1.8; +4.4; P<0.001). The effects on the BDI, AMS, and IIEF-5 were strongest in men with the lowest baseline total testosterone levels (< 7.7 nmol/L, i.e., median value).

Conclusion: TU administration improved depressive symptoms, aging male symptoms and sexual dysfunction in hypogonadal men with the MetS. The beneficial effects of testosterone were most prominent in men with the lowest baseline total testosterone levels.
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EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON MARKERS OF THE METABOLIC SYNDROME AND INFLAMMATION IN HYPOGONADAL MEN WITH THE METABOLIC SYNDROME: THE PLACEBO-CONTROLLED MOSCOW STUDY

Farid Saad, DVM, PhD; Yuliya Tishova, MD, PhD; George Mskhalaya, MD; Louis Gooren, MD, PhD; and Svetlana Kalinchenko, MD, PhD.

Introduction and Objectives: Men with the metabolic syndrome have low plasma testosterone (T) levels and a high-risk profile to develop cardiovascular disease and diabetes mellitus type 2. This study tested whether normalization of plasma T in such men improves features of the metabolic syndrome.

Methods: A randomized, placebo-controlled, double-blind, phase III trial of 184 men suffering from both the metabolic syndrome and hypogonadism. 184 men, 35 to 70 years, with the metabolic syndrome (International Diabetes Federation), and hypogonadism (baseline total testosterone level <12.0 nmol/L or calculated free T level < 225 pmol/L) Treatment for 30 weeks with either parenteral testosterone undecanoate (TU; 1000 mg IM, Nebido®, Bayer Schering Pharma, Berlin, Germany) or placebo, at baseline, and after 6 and 18 weeks. 105 (92.9%) men receiving TU and 65 (91.5%) receiving placebo completed the trial. The following variables were measured: body weight, body mass index (BMI), waist circumference, hip circumference, waist-to-hip ratio, insulin, leptin, glucose, cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, C-reactive protein, interleukin 1 beta (IL-1 beta), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-alpha).

Results: There were significant decreases in weight, body mass index, waist circumference in the TU versus placebo group. Levels of leptin and insulin also decreased, but there were no changes of serum glucose and lipid profile. Of the inflammation markers, IL-1 beta, TNF-alpha, and C-reactive protein decreased, while IL-6 and IL-10 did not change significantly.

Conclusion: 30 weeks of T administration normalizing plasma T improved some components of the metabolic syndrome including a number of inflammatory markers.

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GENISTEIN EXERTS INHIBITION OF HUMAN AND RAT TESTICULAR 3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY

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Introduction and Objectives: Soy-based diets are increasingly being used by the population as non-dairy sources of protein and in individuals with milk allergy. However, soybeans contain soy isoflavones, which are known to regulate testicular steroidogenesis. However, the mechanisms of action in testicular Leydig cells have just begun to receive attention. The objective of the present study was to investigate the effects of genistein and equol on steroidogenic enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) in the human and rat testes.

Methods: Genistein inhibited 3β-HSD activity with an IC50 of 87±15 (human) and 636±155 nM (rat). Thus, human 3β-HSD was more sensitive to genistein-mediated inhibition compared to the rat. The mode of action of genistein on 3β-HSD activity was competitive.

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AN ASSESSMENT OF THE EFFICACY AND SAFETY OF INTRAMUSCULAR INJECTIONS OF 750 MG TESTOSTERONE UNDECANOOATE (TU) IN HYPOGONADAL MEN WITH NO HISTORY OF PRIOR TESTOSTERONE REPLACEMENT THERAPY DURING A 34 WEEK TREATMENT PERIOD

Abe Morgenthaler, MD, FACS; Christina Wang, MD; and Mark Hamett, MS.

Objective: To explore the efficacy and tolerability of TU 750 mg (given every 10 weeks) in hypogonadal men who had not previously been treated with other testosterone replacement therapies (TRTs).

Methods: A multicenter, U.S.-based multicenter study of TU 750 mg in castrator oil solution for intramuscular injection (given at baseline, Weeks 4, 14, and 24). Males, ≥18 years with primary or secondary hypogonadism and screening serum testosterone (T) concentration < 300 ng/dL were eligible. Frequent blood sampling during two consecutive 10-week dosing intervals (injections 3 and 4) was performed to characterize the T replacement. This post hoc analysis describes the cohort of patients enrolled who had never received prior TRTs. The number (%) and 95% confidence interval of patients with average T concentrations (Cavg) within the normal range during a 10-week dosing interval were derived.

Results: 49 patients who were naive to prior TRTs were included; 41 of these patients completed the 34-week treatment period. Mean age was 53.9±1.52 years old; average BMI was 32.6±0.8; and pre-treatment T averaged 238 ng/dL (median: 250.2). Figure 1 provides the average concentration-time profile for T resulting from two injections of TU 750 mg given 10 weeks apart. Average T concentrations remained in the normal range at all time points through the two consecutive 10 week dosing intervals. Ninety-five percent (CI: 88.4, 100%) of patients treated with TU maintained their 10 week T Cavg within the normal range during the dosing interval from Week 14 to Week 34. Adverse events judged by the investigator to be at least possibly related to study treatment were reported in 17 (34.7%) patients. Of these, the most frequently reported was acne, in 3 (6.1%) patients, while PSA increased, irritability, fatigue, hyperhidrosis and injection site pain were each reported in 2 (4.1%) patients, with no other related events reported in more than 1 patient. One patient discontinued the 34-week treatment period due to an AE that was related to study treatment (acne).

Conclusion: Intramuscular testosterone undecanoate appears to be safe and effective in hypogonadal men who have not previously received any form of testosterone therapy.
**Introduction and Objectives:** The impact of testosterone is largely in the brain. However, the effect of testosterone replacement therapy (TRT) on the brains of demented, hypogonadal patients has not been studied.

**Methods:** We studied 12 patients with hypogonadism and dementia. We assessed the impact on cerebral glucose metabolism (CGM) during a mental rotation task via (18F) fluorodeoxyglucose (FDG)-positron emission tomography (PET).

**Results:** Selected results from two subjects are presented here. Subject A has moderate dementia (MMSE = 21). Subject B has severe dementia (MMSE = 0). Under testosterone replacement therapy, enhanced cerebral glucose metabolism was observed in these subjects in two relevant brain regions.

<table>
<thead>
<tr>
<th>Structure Subject</th>
<th>Baseline</th>
<th>Treatment</th>
<th>p value</th>
<th>Anatomical</th>
</tr>
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<tbody>
<tr>
<td>Function z-score</td>
<td>z-score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Word meaning, face recognition</td>
<td>A</td>
<td>-9.84</td>
<td>-2.96</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Medial occipital gyrus A</td>
<td>B</td>
<td>-7.76</td>
<td>-3.59</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
| z-score: Deviation from the mean of a sample of age-matched individuals. Results of neurocognitive testing were unremarkable. This is principally due to the fact that the tests selected for this pilot study were not sensitive to change in the study population ultimately included. Further study is needed in a mildly demented population.

**Conclusion:** Findings as to areas of the brain that demonstrate improved CGM under TRT are consistent with a study done in non-demented, hypogonadal men(1). Improvement in functional outcomes associated with these areas could have significant impact on quality of life. This pilot study should be expanded and more work done to assess functional outcomes such as cognitive function, activities of daily living, and caregiver stress.

**Funding:** Solvay Pharmaceuticals


**ANTIOXIDANT EFFECTS OF INDIAN MEDICINAL PLANTS ON SEMINAL PLASMA ENZYMES OF HIGH GLUCOSE FED RATS**

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**Introduction:** Oxidative stress (OS) due to hyperglycemia has been suggested in semen of diabetic male. Brahmi (Bacopa monniera, an Indian medicinal plant) and Triphala (a traditional Indian ayurvedic medication composed of equal parts of three different plants: Terminalia chebula, Terminalia belerica, and Emblica officinalis) have been shown to have significant antioxidant property.

**CLOMIPHENE CITRATE TREATMENT FOR IDIOPATHIC ADULT ONSET HYPOGONADOTROPIC HYPOGONADISM OVER ONE YEAR**

George Toth, MD

**Introduction:** Clomiphene (Clomid) is a Selective Estrogen Receptor Modulator, which can block estrogen receptors at the hypothalamus: pituitary axis, increasing LH & FSH that subsequently stimulate Leydig cell testosterone and Sertoli cell sperm production. Although Clomid is widely used in female in-vitro fertilization protocols, there are very few studies examining its long-term application for Idiopathic Adult Onset Hypogonadotropic Hypogonadism (IAHH).

**Aim:** To study Clomid's potential for long term treatment of IAHH in an observational study over 1 year.

**Methods:** A 45-year-old male diagnosed with IAHH previously on Androgel for 1 year prior to study, was put on Clomid 25mg daily for 1 year. Regular labs were obtained using Labcorp and subjective response was evaluated using the Androgen Deficiency in Aging Male Questionnaire (ADAM) score every 3 months. Inhbin-B was used as a surrogate marker for Sertoli cell function.

**Results:** LH and FSH rose immediately after stopping Androgel and commencing Clomid with the commensurate elevation of Testosterone that was sustained around 700 ng/dl throughout the study. (See Figure 1.) At day 240, the patient developed blurry vision necessitating an interruption of treatment for 3 weeks. This resulted in a drop of Testosterone, LH and FSH, which promptly rebounded upon the resumption of Clomid. Subjective symptoms as measured on ADAM showed that Clomid maintained energy, erection strength and work performance but had decreased libido and mood as compared to Androgel. Throughout the study the FSH and LH consistently trended down over time, indicating a decreased effectiveness perhaps from tolerance or receptor downregulation. Inhibin-B was steady at 200 pg/mL indicating Sertoli cell function was maintained after 1 year.

**Conclusion:** Clomiphene can be used effectively in IAHH for short periods of time with improved Testosterone and subjective results as measured on the ADAM questionnaire. The long term side effects and consistent decline in gonadotropin efficacy over time need further study. Finally, even at the peak Testosterone levels, the patient's libido and mood did not approach the previous levels the patient had experienced with Androgel. Further research is needed.
Aim: Our aim was to evaluate the effects of Triphala, Brahmi as well as combination of Triphala and Brahmi on activities of the catalase and glutathione peroxidase (antioxidant enzymes) as well as activity of sorbitol dehydrogenase (SDH) in seminal plasma of high glucose fed rats.

Materials and Methods: The rats were divided into 8 groups containing 6 rats in each group (Table 1). All rats were given lab feed and water ad libitum. Rats in glucose diet group were given high glucose diet consisting of 50 grams glucose added to each 100 grams of feed. The rats in Brahmi (or Triphala or Brahmi and Triphala groups) were orally administered Brahmi (and/or Triphala) powder (3.6 mg/100g body weight/day) dissolved in 0.9% normal saline during every morning for 30 days. After 30 days feeding the weights of the rats were taken and deprived of food overnight. The next day the rats were sacrificed. Enzymes activities were measured in seminal plasma collected from seminal vesicle.

Results: Our result showed decreased activity of catalase in high glucose fed rats; increase in activity of antioxidant enzymes and decrease in activity of SDH in semen after Triphala and Brahmi administration. Triphala-Brahmi combination had higher effect than Triphala or Brahmi alone.

Conclusion: Hyperglycemia increases enzymatic conversion of glucose to sorbitol, which leads to decrease in NADPH and glutathione. High glucose feeding also leads to OS by mitochondrial generation of superoxide, which is converted into hydrogen peroxide by SOD. Catalase is an important primary scavenger enzymes involved in detoxifying hydrogen peroxide in mammalian systems. Increase in activity of catalase and glutathione peroxidase while decrease in activity of SDH after Triphala and Brahmi administration suggests beneficial role of these herbs on hyperglycemia induced OS.
**Results:** Our results indicate that C/EBP and c-Myc may participate in the transcriptional suppression of testis TIMP-2 following MEHP exposure. The addition of follicle-stimulating hormone (FSH) rescues MEHP-suppressed TIMP-2 levels and causes the translocation of CREB and C/EBP into the nucleus. The down-regulation of TIMP-2 expression by MEHP exposure is blocked by adding 8-bromo-cAMP (a cAMP analog) or forskolin (a cAMP-elevating agent) in a dose-dependent manner in vitro.

**Conclusion:** These observations suggest that the decrease in Sertoli cell TIMP-2 by MEHP exposure is cAMP-dependent. Treatment with TIMP-2 significantly suppressed MEHP-induced germ cell sloughing, indicating that TIMP-2 plays a primary role in this process. Taken together, the participation of FSH-stimulated cAMP in controlling TIMP-2 expression is likely suggested to be the consequence of decreased Sertoli cell support in response to MEHP exposure. The decrease in TIMP-2 levels in Sertoli cells is the key to lead to abnormal spermatogenesis.

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**COMPARISON OF THE POTENCIES OF PERFLUOROALKYLATED SUBSTANCES INHIBITING 3β- AND 17β-HYDROXYSTEROID DEHYDROGENASE 3 ACTIVITIES IN HUMAN AND RAT TESTES**

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(Presented By: Binghai Zhao, PhD)

**Introduction and Objectives:** Perfluoroalkylated substances (PFASs) including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been classified as persistent organic pollutants and endocrine disruptors causing reduced testosterone production in males. The objective of the present study was to compare the potencies of five different PFASs including PFOA, PFOS, potassium perfluorooctylsulfonate (PFOSK), potassium perfluorohexanesulfonate (PFHxSK) and potassium perfluorobutane sulfonate (PFBSK) in the inhibition of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxyysteroid dehydrogenase 3 (17β-HSD3) in human and rat testes.

**Methods:** Either human or rat microsomal enzyme was subjected to the exposure to various PFASs. PFOS and PFOSK inhibited rat 3β-HSD with IC50s of 1.35±0.05 and 1.77±0.04 µM, respectively, and PFHxSK and PFBSK did not inhibit the enzyme at concentration up to 250 µM. However, all these chemicals weakly inhibited human 3β-HSD with IC50s over 250 µM. PFOS, PFOSK and PFOA inhibited human 17β-HSD3 with IC50s of 6.02±1.02, 4.39±0.46 and 127.60±28.52 µM, respectively. The potencies of inhibiting 17β-HSD were PFOSK > PFOS > PFOA for PFHxSK-PFBSK for human 17β-HSD3.

**Results:** There was a species difference in the sensitivity to PFAS-mediated inhibition of 17β-HSD3. For example, the IC50s of inhibiting 17β-HSD3 for PFOS(K) were over 250 µM. PFOS(K) showed competitive inhibition of rat 3β-HSD and human 17β-HSD3.

**Conclusion:** In conclusion, the present study shows PFOS and PFOSK are potenti inhibitors of human 11β-HSD3 and there are structure- and species-dependent differences for the potency inhibiting 17β-HSD3.

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**ESTROGEN INHIBITS SPERMATOGENESIS IN PUBERTAL MICE BY BLOCKING TESTICULAR DESCENT AND SUPPRESSING TESTOSTERONE**

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(Presented By: Gunapala Shetty, PhD)

**Introduction and Objectives:** To investigate the reported strain differences in testicular sensitivity of mice to estrogen exposure.

**Methods:** We treated the pubertal and adult C3H, B6, and 129 mice for a period of 3 weeks with two different doses of estradiol (E2) given by subcutaneous implants. The 3-week E2 treatment was given to pubertal mice starting at different times during postnatal days (PND) 18-30, and to adult mice starting at 8 weeks of age. In the pubertal mice the B6 mice were most sensitive to E2-induced suppression of sperm head count (SHC) and the 129 mice were least sensitive. The time window of the initiation of E2 treatment required to cause significant reduction in SHC was PND18-25, 18-21, and 18-19 in B6, C3H and 129 mice, respectively. No suppression of SHC was observed in any of the strains when E2 treatment was started at PND 30 or later. Interestingly, in all the above cases severe suppression of SHC was associated with complete inhibition of testicular descent during treatment. The intratesticular testosterone (ITT) levels were reduced by E2 in pubertal mice of all strains, slightly to a lesser extent in 129 mice. In adults greatest suppression of ITT was observed in B6 mice with no suppression in 129 mice. To distinguish the effects of E2-induced suppression in ITT and blocking testicular descent on SHC reduction, we performed two experiments treating pubertal B6 mice starting at PND 21. First, testosterone (T) was administered in two different doses in combination with E2. Testes were still undescended, suggesting direct inhibitory action of E2 on testicular descent in pubertal mice; nevertheless T dose-dependently reversed the suppression of SHC by E2. Secondly, the mice were cryptorchidized with or without E2 treatment. SHC was only marginally reduced by cryptorchidism alone, but was markedly reduced by the combination with E2.

**Results:** Thus, the severe suppression of spermatogenesis in pubertal mice is a result of the combination of cryptorchidism and suppression of T. The strain specific differences in E2 effects on spermatogenesis is determined by the differences in the degree of T suppression, the sensitivity of the testicular descent to E2, and the sensitivity of germ cells to elevated temperatures encountered in the low abdominal region.

**Conclusion:** The deleterious effect of E2 on spermatogenesis is restricted to a specific pubertal period; once the testicular descent is fully complete, the mouse testes appear to be resistant.

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**EFFECTS OF METHOXYCHLOR AND 2,2-BIS(P-HYDROXYPHENYL)-1,1,1-TRICHLOROTHANE ON 3β-HYDROXYSTEROID DEHYDROGENASE AND 17β-HYDROXYSTEROID DEHYDROGENASE 3 ACTIVITIES IN HUMAN AND RAT TESTES**

Xiao-Heng Li, MS, Binghai Zhao, MD, Yanhui Chu, PhD, Benson T. Akingbemi, MD, Zhi-Qiang Zheng, MD, Ren-Shan Ge, MD and Guo-Xin Hu, MD

(Presented By: Xiao-Heng Li MS)

**Introduction and Objectives:** Human and rat testis microsomes were used to investigate the direct inhibitory activities of methoxychlor (MXC) and its metabolite 2,2-bis(p-hydroxyphenyl) 1,1,1-trichloroethane (HPTE) on 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) in human and rat testes.

**Methods:** MXC and HPTE inhibited human 3β-HSD activity at a concentration of 10 nM. The IC50s for MXC inhibition of 3β-HSD were 53.21±15.52 µM (human) and 46.15±17.94 µM (rat), and for HPTE was 8.29±2.49 µM (human) and 13.82±2.26 µM (rat).

**Results:** At the higher concentration of 100 µM, MXC did not affect human and rat 17β-HSD3 activity. However, the IC50s for HPTE inhibition of 17β-HSD3 were 12.1±1.9 µM (human) and 32.0±8.6 µM (rat). The mode of action of MXC and HPTE on 3β-HSD activity was noncompetitive with the substrate pregnenolone but was competitive with the cofactor NADPH. The mode of HPTE inhibition of 17β-HSD3 was noncompetitive with the substrate androstenedione but was competitive with the cofactor NADPH.

**Conclusion:** In conclusion, HPTE, which is the biologically active metabolite of MXC, has the capacity for direct inhibition of 3β-HSD and 17β-HSD3 enzyme activity. The inhibitory activity is linked to suppression of steroidogenesis in gonadal tissues, which has been reported for MXC.
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SHORT-TERM CIGARETTE SMOKE EXPOSURE CAUSES OXIDATIVE STRESS IN LEYDIG CELL AND INCREASES GERM CELL APOPTOSIS
Riyad Ellati, MD and Jeffrey Lysiak, PhD
Department of Urology, University of Virginia
(Presented By: Riyad Ellati, MD)

Introduction and Objectives: Smoking constitutes an environmental risk factor that has a detrimental impact on numerous tissues of the body. Male smokers constitute about one billion of the world population, 35% in developed countries and 50% in developing countries. Many studies suggest the correlation between smoking and the production of reactive oxygen species in humans, rats and isolated cells. Several studies have found that long-term smoke exposure can be detrimental for spermatogenesis.

Methods: The present study investigated the effect of short-term smoke exposure on oxidative stress and apoptotic cell death in the tests. Mice were divided into four groups: group 1, was exposed to cigarette smoke with total suspended particle of 90mg/m3 and CO of 350 ppm for 6 hours/day/5 days a week, for 3 weeks. Group 2, remained in room air in the same room as animals in group 1. Group 3, testes were injected with hydrogen peroxide 125 mM and testes were harvested 24 hours later. Group 4, was the same as 3; however, saline was injected. Immunostaining for a marker of lipid peroxidation revealed that Leydig cells in groups 1 and 3 were positive for oxidative stress, while little or no staining was seen in groups 2 and 4.

Results: Staining for apoptotic cells revealed that there was an increase in apoptotic germ cells in groups 1 and 3 compared to the other groups. Other cell types in the testis showed no apoptotic staining.

Conclusion: Based on these observations we can conclude that even short term exposure to cigarette smoke can have detrimental effects on both Leydig and germ cells in the tests. We are currently investigating if the increase in germ cell apoptosis seen in mice exposed to cigarette smoke is directly linked to the smoking-induced oxidative stress observed in the Leydig cells or if they are two separate pathologies.

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GENOME-WIDE APPROACHES TO IDENTIFYING SPERM BIOMARKERS OF TOXICANT EXPOSURE
Sara Pacheco, BS, Christoph Schorl, PhD, E. Andres Houseman, PhD, Karl Kelsey, MOH, MD, Mark Sigman, MD and Kim Boekelheide, MD, PhD
Brown University
(Presented By: Sara Pacheco, BS)

Introduction: The reproductive function of men is susceptible to a variety of occupational and environmental toxicants. An obvious undesirable consequence of long-term exposure to these reproductive toxicants is infertility.

Objectives: The goal of this project was to use rodent exposure models and human samples to develop the microarray techniques required to identify sperm biomarkers of effect.

Methods: Rats: Groups of adult male Fischer rats were chronically exposed to either 0.33% 2,5-hexanedione (HD) (N=6), 5 mg/kg/d 1,2-dibromo-3-chloropropene (DBCP) (N=10), or their respective controls: water (N=10) and corn oil (N=10), for 12 weeks. For each exposure, caudal sperm mRNA was isolated from multiple pools of two rats and these samples were classified into three groups (control, HD exposed, and DBCP exposed), with a total of 10, 3, and 5 pooled samples, respectively. The 18 resulting samples were processed and hybridized to Affymetrix GeneChip Rat Gene 1.0 ST arrays. Humans: Sperm DNA was isolated from 6 men presenting for fertility evaluation. Prior to microarray analysis the DNA was bisulfite modified. Samples were then processed and run on the Illumina Infinium Methylation array to measure genome-wide methylation changes.

Results: Rats: Differential gene expression was detected between toxicant and control mRNA. Comparing HD versus control, clusterin was the only gene identified as significant (1.7 fold increase; 3.3 log-odds) after adjusting for false discovery rate. We also investigated the localization and function of the other differentially altered genes using Ingenuity Pathway Analysis software and literature searches. This analysis revealed 3 genes that are known to be sperm-specific: testis fascin (Fascn3), aldehyde oxidase (Aox3) and A kinase anchor protein (Akap1). Humans: Recursively partitioned mixed modeling (RPM) clusters differentiate whole blood from sorted lymphocytes and differentiate sperm from both of these. Overall, sperm has a tendency to be less methylated than blood. Importantly, we can detect differences between sperm of individual men.

Conclusion: We validated the techniques required for future biomarker identification in larger scale studies and determined that sperm gene expression and DNA methylation are sensitive biomarkers of effect.

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ENVIRONMENTAL ORGANOCHLORINES AND SEX CHROMOSOME DISOMY IN HUMAN SPERM
Melissa J. Perry, ScD, MHS¹, Xing Chen¹, Megan McAuliffe¹, Arnab Maity², Glen Deloid³ and Larisa Altshul⁴
¹Department of Environmental Health, Harvard School of Public Health; ²Department of Biostatistics, Harvard School of Public Health
(Presented By: Melissa J. Perry, ScD, MHS)

Introduction: Although organochlorines such as polychlorinated biphenyls (PCBs) and DDT (dichlorodiphenyltrichloroethane) have endocrine disrupting properties and have been associated with inferior sperm parameters, no human studies have evaluated whether they are associated with sperm aneuploidy.

Objective: To investigate whether environmental exposures to PCBs and dichlorodiphenyltrichloroethylene (DDE) are associated with sex chromosome disomy in human sperm.

Methods: We used multi-color fluorescence in situ hybridization to measure the rate of sperm with numerical sex chromosome abnormalities among 60 men attending a large urban U.S. hospital based fertility clinic. Serum PCB ranged from 61.3 to 1590 ng/g lipid, and serum p,p'-DDE ranged from 34.8 to 6152 ng/g lipid. Men were categorized separately into PCB and p,p'-DDE exposure quartiles, and X and Y disomy rates were compared using quartile 1 as the reference.

Results: After adjusting for age, sperm concentration, smoking status and abstinence time, we found an association with XY disomy in the highest p,p'-DDE exposure quartile (rate ratio (95% CI) = 1.88 (1.08-3.25)). After removing four outliers and adjusting for the same important covariates, we saw p,p'-DDE dose-dependent increases in the rate of total disomy, XX disomy and XY disomy (trend p<0.04). We did not see comparable evidence of an association with PCB.

Conclusion: p,p'-DDE exposure is associated with increased rates of sex chromosome sperm disomy and should be investigated further for its aneuneic properties.

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REVERSIBLE SUPPRESSION OF SPERMATOGENESIS BY THE BIS-DICHLOROACETYLIDIAMINE WIN 18,446 IS MEDIATED BY INHIBITION OF RETINOIC ACID ACTIVITY WITHIN THE TESTES
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¹University of Washington; ²Washington State University; ³Focused Scientific
(Presented By: John Amory, MD, MPH)
Introduction and Objectives: The bis-dichloroacetyldiamine WIN 18,446 reversibly inhibits spermatogenesis in man; however, the mechanism by which WIN 18,446 functions is unknown. As spermatogenesis depends on retinoic acid, we hypothesized that WIN 18,446 might act by inhibiting retinoic acid activity within the testes.

Methods: We studied the effect of WIN 18,446 on testicular histology, spermatogenesis and the expression of the retinoic acid regulated gene Stra8 in New Zealand white rabbits. Animals were dosed orally with 200 mg/kg WIN 18,446 daily for 4, 8 or 16 weeks (n=4/group), at which time animals underwent hemi-orchidectomy for histology and measurement of Stra8 mRNA. In the animals treated for 16 weeks, sperm was collected weekly during treatment and for twenty weeks thereafter using an artificial vagina. In addition, these animals underwent mating trials to assess fertility.

Results: Oral administration of WIN 18,446 severely suppressed spermatogenesis during treatment, with all animals achieving a sperm concentration of less than 1 million sperm/ml after 16 weeks of treatment (P<0.001 compared with baseline). In addition, Stra8 mRNA expression was reduced by more than 70% compared with baseline (P<0.01 compared with baseline). Testicular histology revealed only spermatagonia and Sertoli cells. Testicular volume was significantly reduced, but epididymal weights were unaffected. Sperm concentrations recovered after treatment was discontinued. In addition, there were no significant changes in animal behavior, blood counts, serum chemistries or serum testosterone concentrations. Animals were unable to father pregnancies at the end of treatment, but had normal fertility after recovery of spermatogenesis.

Conclusion: Oral administration of WIN 18,446 safely and reversibly inhibits spermatogenesis in rabbits. This effect appears to be mediated by inhibition of retinoic acid activity in the testes as inferred by Stra8 expression. Bis-dichloroacetyldiamines such as WIN 18,446 may have promise as safe and effective oral, non-hormonal, reversible contraceptives in man.

Funding: This work was supported by the Eunice Kennedy Shriver National Institute of Childhood Health and Human Development grant #U01HD060408.

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GHERLIN PREVENTS CISPLATIN-INDUCED SPERM DNA DAMAGE
Jose Garcia, MD1, Shannon Whirledge, PhD2, Victor Papusha, BS3, Tripti Halder, BS3, Roy Smith, PhD2 and Dolores Lamb, PhD2
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(Presented By: Jose Garcia, MD)

Introduction: Cisplatin, achemotherapeutic agent commonly used for the treatment of many cancers, is known to cause testicular damage and germ cell apoptosis by inducing DNA cross links. As a result of this, more than 50% of male cancer patients subjected to cisplatin-based chemotherapy will suffer from long-term infertility. However, medical treatment for chemotherapy-induced infertility is not currently available. We have previously shown that ghrelin, a novel hormone, prevents cisplatin-induced germ cell apoptosis in the testis. However, its effects on DNA integrity are not known.

Objectives: Determine the effects of cisplatin and ghrelin on germ cell apoptosis and sperm DNA integrity.

Methods: Young adult C57bl/6J mice (n=4/group) were treated with vehicle (V), cisplatin (C), ghrelin (G) or ghrelin + cisplatin (GC). Testicular apoptosis was assessed by flow cytometry and confirmed by TUNEL assay. Sperm DNA quality was assessed by the Comet assay.

Results: Cisplatin induced an increase in germ cell apoptosis and this was partially prevented by ghrelin coadministration (Figure 1). Sperm DNA damage was significantly increased by cisplatin when compared to vehicle and this was prevented by ghrelin co-administration (COMET-positive cells 1.7±0.43%, 4.67±2.6%, 10.2±4.1% and 2.83±1.6% for vehicle, ghrelin, cisplatin and cisplatin+ghrelin respectively; p<0.05).

Conclusion: Ghrelin prevents cisplatin-induced testicular germ cell apoptosis and sperm DNA damage. Prevention of the secondary infertility due to damage to the seminiferous epithelium and sperm by ghrelin would be a significant advance for patients facing chemotherapy for their malignancies.

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AN EVALUATION OF MALE FACTOR AS A CONTRIBUTING FACTOR TO UNEXPLAINED INFERTILITY
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*UMDNJ, Robert Wood Johnson Med. School at Camden
(Presented By: Brittney Katsoff, BA)

Introduction and Objectives: For couples with unexplained infertility and male partners with normal semen parameters, there is the possibility that there may be subtle sperm defects. This could lead to failed fertilization or the creation of embryos with markedly reduced implantation potential. It was hoped that more insight on the role of male factor in unexplained infertility would be provided by comparing pregnancy and fertilization rates in couples with unexplained infertility when the female partner of the couple shared half of her oocytes with recipients with diminished egg reserve (as their cause of infertility).

Methods: A retrospective comparison of fertilization and pregnancy rates in infertile donors with unexplained infertility vs. recipients of their shared oocytes was performed. Couples were given the option of ICSI vs. conventional oocyte insemination after being advised of the advantages and disadvantages of each procedure.

Results: Twenty-one donor-recipient pairs were evaluated. For donors 16 chose conventional insemination vs. 9 for recipients (the choice of ICSI was usually for male factor in recipients). Twelve donors and 15 recipients had fresh embryo transfers. For donors, two transfers did not occur because of failed fertilization with conventional oocyte insemination and 9 were for risk of ovarian hyperstimulation syndrome where cryopreservation of embryos was performed. For recipients the 6 cancelled embryo transfers were for the creation of embryos with markedly reduced implantation potential. It was hoped that more insight on the role of male factor in unexplained infertility would be provided by comparing pregnancy and fertilization rates in couples with unexplained infertility when the female partner of the couple shared half of her oocytes with recipients with diminished egg reserve (as their cause of infertility).

Conclusion: An occult male factor does not seem to be a common cause of producing embryos that are not likely to implant. If this were so one may have expected a low pregnancy rate in the donors with unexplained infertility but not so in their respective recipients. Instead a very high pregnancy rate was found. However, failed fertilization in about 12.5% of the cases may be found. Normal fertilization in some instances by the recipient’s male partner using conventional insemination suggests that failed fertilization is more likely related to a sperm defect rather than an oocyte abnormality.

OOCYTE QUALITY INFLUENCES THE EFFECT OF SPERM DNA FRAGMENTATION ON PREGNANCY CHANCES, AS DEMONSTRATED BY LOGISTIC REGRESSION ANALYSIS
Rebeca Santiso, PhD1, Nicolas Garrido, PhD2, Jose L. Fernandez, PhD3, Sandra García-Herrero, PhD4, Thamara Vitoria, PhD3 and Marcos Meseguer, PhD5
*21NIB-Genetica, Hospital “Teresa Herrera,” Complejo Hospitalario Universitario A Coruña, 15006-A Coruña, Spain; IVI Valencia, Spain
(Presented By: Marcos Meseguer, PhD)

Introduction and Objectives: Several bias factors can be masking the real effect of Sperm DNA fragmentation (SDF) on reproductive success. Our objective was to quantify this effect by logistic regression analysis on reproductive outcome, in sperm samples from patients undergoing IVF or ICSI with fresh (F) or frozen-thawed (T) sperm, with oocytes coming from infertile patients (I) and from fertile young donors (D).

Methods: A prospective cohort study on first cycles were performed on sperm (before and after swim-up) used for IVF or ICSI with F or T sperm with I and D oocytes (320 samples, 160 cycles). SDF was determined by the improved SCD test, using the Halosperm kit (Halotech DNA, Spain). Odds ratio (OR) of the effect of SDF on pregnancy was expressed together with 95% confidence interval (CI95) and significance. In the regression analysis, a stratified test was performed in order to consider oocyte quality like a bias factor (I or D), possible confusions factors were: fertilization procedure and sperm origin (F or T). These factors were introduced by forward step method. By doing this procedure we checked the confusion effect of these variables on pregnancy. In the computer analysis those variables which p value is >0.2 were introduced progressively. After the statistical analysis, the variables can be classified as those which did or did not bias importantly (that is, less that 10% of variation in the OR) the effect of SDF on pregnancy outcome.

Results: Swim-up decreased SDF from 36.83% (SD=1.60) to 15.84% (SD=1.60) (p<0.001). The effect of SDF on pregnancy chances was not affected by the sperm origin (F or T) and the fertilization procedure (IVF or ICSI) in both cases (before and after swim-up), in consequence they are not considered as bias factors and were not included in the model. The effect of SDF on pregnancy chances was quantified as OR=0.973 (CI95 0.948-0.999) (p<0.01) in I oocytes. Otherwise, OR=0.991 (CI95 0.971-1.01) (p>0.05) when D oocytes were considered. No important effect was observed when SDF was analyzed after swim-up in both cases (I or D oocytes).

Conclusion: The consequence of SDF on pregnancy chances can be calculated independently of the fertilization procedure (IVF or ICSI) or sperm origin (F or T). Outstandingly, oocyte quality is conditioning the effect of SDF, since it was significant when oocytes came from I, whereas it was not evidenced when oocytes come from D. Furthermore, the analysis of SDF after swim-up is not clinically useful.

WHY MEN DO NOT USE THEIR CRYOPRESERVED SEMEN
Marique Sorel, Eline Zijltop and M.T.W.T Lock
(Presented By: Marique Sorel)

Introduction and Objectives: Men with testicular cancer are at risk of becoming infertile from cancer therapy (1). The chance for spontaneous reproduction after chemotherapy is difficult to predict. The only established method to increase fertility is semen cryopreservation (SCP). The rate of referral for assisted reproductive techniques (ART) of those who have banked their semen is low (3.6-16.7%) (2). We evaluated the reasons why patients do not use their cryopreserved semen, to avoid unnecessary semen banking in the future.

Methods: A 17-year cryopreservation programme, involving 97 men with testicular carcinoma who preserved their semen before proceeding therapy, was reviewed. Data regarding cancer diagnosis, age and use of frozen semen were analyzed.

Results: Of 303 patients, diagnosed with testicular cancer between 01-01-1990 and 31-12-2007, 97 banked their semen (32.0%). The median follow-up time after SCP was 6.3 years (range: 1-17 years). Three patients died during follow-up, 19 patients were lost to follow-up and 5 couples requested the banked semen. The remaining 70 patients (97-27 patients) did not use their semen. Out of these 70 patients, 27 patients (37%) had no child wish; 21 patients assumed they were too young, 1 patient thought he was too old, 3 patients had already completed their family before the treatment of the tumor and 2 patients did not want children. A spontaneous pregnancy was reported in 26 of the 70 patients. 14 patients had no partner. One partner of a patient had infertility problems and 2 patients thought the fertility treatment would be too invasive.

Conclusion: Out of the 97 patients who banked their semen, a majority did not use it (72%). Several reasons can be summed up: patients believed they were too young, patients reported a spontaneous pregnancy or they had no partner. A much longer follow-up is needed to determine factors to predict the use of banked semen.

DOES MORPHOLOGY IN SEMEN PARAMETERS INFLUENCE REPRODUCTIVE OUTCOMES IN PATIENTS UNDERGOING IUI CYCLES?
Fernando Quintana, BScEd, Iratxe Penalba, BScEd, Zaloa Larreategui, BScEd, Fernando Ayerd, BScEd, Guillermo Quea, MD and Jose Serna, MD, PhD
(Presented By: Fernando Quintana, BScEd)

Objective: It is widely accepted that Intrauterine Insemination (IUI) with ovarian stimulation plus induction of ovulation has become the first line of treatment for infertility of unknown origin. The aim of this study was to determine the effect of morphology as a seminal parameter in order to evaluate reproductive success in patients undergoing IUI in our facilities.

Materials and Methods: Retrospective study including 438 couples with unexplained infertility, undergoing IUI cycles. The period of the study ranges from December 2005 to September 2009. All patients were stimulated with rFSH (Peregou; Organon) starting cycle day 3, once ovarian quiescence was confirmed by transvaginal ultrasound scan, and estradiol and progesterone blood tests when needed. Starting dose ranged between 75 and 150 IU, depending on patients' age and BMI. Final maturation was triggered with 250 µg of rhCG when at least one follicle reached 17 mm in mean diameter. Two IUI were performed at 16 and 28 hours after rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, by masturbation after a minimum of two days of abstinence. KrugerA’s strict criteria were applied to evaluate sperm morphology. According to percentage of normal forms, samples were classified into Group A (1-6% normal sperms) and Group B (7-14%). Pregnancy Rate (PR) and Miscarriage Rate (MR) were compared between both groups. t-test was applied for statistical analysis.

Results: Although there is a trend towards better outcomes with increasing number of normal sperm, there were no statistically significant differences between both groups in terms of PR [A: 21.03% (82/390); B: 29.17% (14/48)] and MR [A: 2.31% (9/390); B: 2.08%, (1/48)].

Conclusion: Sperm morphology is a widely used parameter to consider IUI. Our results indicated that, at least in our facilities, it does not predict IUI outcomes in terms of PR and MR. The narrow range of sperm morphology classification may be responsible of these results, although WHO criteria to classify morphology seems to show similar results. In the view of these results, there is no clinical usefulness of morphological classification at least to predict PR and MR.

BIOLICAL PROCESSES (BP) STATISTIACALLY AFFECTED (SA) IN FRESH SPERM SAMPLES (SS) FROM FERTILE DONORS (D) VS. INFERTILE MALE PATIENTS (IP) WITH A REPRODUCTIVE ROLE (RR) RELATED
Nicolás Garrido, PhD1, Sandra García-Herrero, PhD2, M. Angeles López-Hervás, PhD3, Inmaculada Pérez-Can, PhD3, Jose-Antonio Martínez-Conejero, PhD4 and Marcos Meseguer, PhD5
1IVI Foundation; 2IVI Valencia; 3IVI Alicante; *Genomix
(Presented By: Nicolás Garrido, PhD)

Introduction: Male fertility evaluation is usually focused on basic SS analysis, remaining molecular factors as mRNA presence within ejaculated spermatozoa. Our group defined a list of GDE from patients achieving pregnancy (group P 1568 GDE) compared with those who didn’t (group NP 1642 GDE) by microarray technology.

Objective and Design: Study's aim was to analyze by means of bioinformatic tools these lists searching for BP affected in both groups pointing those with a RR. SS were obtained from IP (n=5), and D (n=5), both groups presenting normal sperm count and motility (WHO criteria) parameters. Transcript profile from both groups was assessed on the short-oligonucleotide-based microarrays with 55000 reporters.

Material and Methods: Sperm mRNA was extracted using Trizol protocol RNAs and was analyzed on Agilent Bioanalyzer 2100. GDE lists were analyzed by DAVID genes bioinformatics tool (http://david.abcc.ncifcrf.gov/) to detect BP SA.

Results: In D group we found 5 BP SA with a RR and between brackets % of GDE described in BP regarding total number of genes described in that BP in H. sapiens as reproduction (45.3%), gamete generation (28.6%), sexual reproduction (29.83%), male gamete generation (31.96%) and spermatogenesis (31.96%) whereas in group IM we don't find any BP reproductive related SA.

Conclusion: Is very noteworthy that no RR BP was present in IP group and present in D group, so we can think that these BP are more operational in D, therefore this faction has more reproductive chances. Furthermore, bearing number of GDE implicated in a BP respect total number of genes involved in H. sapiens in that BP, we are getting used to the idea of how much is affected in that BP. Despite we list hundreds of GDE between both groups, those genes by themselves can't explain differences, we have to correlate them with their biological function so as important as to list GDE between two or more biological situations as fertile vs. infertile is to interpret the biological meaning.

PERCUTANEOUS EMBOLIZATION VARICOCELES. NUMBER ONE TREATMENT? (10 YEARS OF FOLLOW-UP)
Marique Sorel, A.A.G.M. Giesbers and P.L.M. Vijverberg
(Presented By: Marique Sorel)

Introduction: There are different treatment options for a varicocele. The Dutch and European guidelines indicate no preferential treatment; it depends on the experience of the urologist. A percutaneous embolization (PE) is a minimally invasive treatment without the risk of arterial damage and hydrocele formation. In our hospital PE is the preferential treatment since 1998. The aim of this study is to evaluate the results of PE and compare results with literature.

Methods and Materials: Between 1998 en 2009 55 patients with an (a) symptomatic, left-sided varicocele underwent PE. Follow-up was possible in 45 patients. Retrospective chart review and/or telephonic contact was performed. Treatment results and morbidity were registred.

Results: We performed 60 PE of which five patients underwent 2 PE. Technical failure to place coils occurred in two patients and in 3 patients there was no retrograde blood flow in the internal spermatic vein. The varicocele persisted in 2 patients: 1 of them had successful repeat PE. One patient underwent a second PE, which was successful. Recurrence occurred in 5 patients (range 2-48 months). If they underwent a second PE, this was successful. Of the 36 symptomatic varicocele, in 4 patients the symptoms remained unchanged after PE. During intervention complications occurred in 4 (7%) patients and there were no post-intervention complications.

Conclusion: The results of percutaneous embolization are better than surgical techniques (open or laparoscopic) and there are less complications, compared to literature. A second percutaneous embolization can be considered if first percutaneous embolization is not successful.
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