MARCH/APRIL 2009
Supplement

Journal of ANDROLOGY

American Society of Andrology

34th Annual Meeting

April 4–7, 2009
Philadelphia, Pennsylvania

Program and Abstracts

Published by THE AMERICAN SOCIETY OF ANDROLOGY

schedule at a glance

34th ASA Annual Meeting
April 4 – 7, 2009

XX North American Testis Workshop
April 1 – 4, 2009

Andrology Lab Workshop
April 4 – 5, 2009

ASA Special Symposium
April 4, 2009

34th Annual Meeting
"Bench to Bedside: Advances in Andrology"
April 4 – 7, 2009

Program Chairs: Dolores Lamb, PhD
Dana Ohl, MD

*All sessions will be located in **Columbus Ballroom** unless otherwise noted.*
Registration fee includes entry into the lectures, one ticket to the Welcome Reception, a syllabus, and refreshment break.

FRIDAY, APRIL 3, 2009

7:00 a.m. – 6:00 p.m.	**Registration** *Location: Columbus Ballroom Foyer, 2nd Floor*

SATURDAY, APRIL 4, 2009

7:00 a.m. – 7:00 p.m.	**Registration** *Location: Columbus Ballroom Foyer, 2nd Floor*
4:00 p.m. – 9:30 p.m.	**Exhibit Hall Open** *Location: Grand Ballroom, 2nd Floor*
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.	**ASA KEYNOTE LECTURE** **Sex Determination and Fetal Testis Development** *Blanche Capel, PhD* *Duke University School of Medicine* *(Introduced by Wayne J.G. Hellstrom, MD)*
7:30 p.m. – 7:45 p.m.	**Updates from NICHD** *Stuart Moss, PhD* *NICHD*
7:45 p.m. – 9:30 p.m.	**Welcome Reception** *Location: Grand Ballroom, 2nd Floor*

SUNDAY, APRIL 5, 2009

6:30 a.m. – 8:00 a.m.	**Past President's Breakfast** *Location: Riverview A, 3rd Floor*
7:00 a.m. – 4:00 p.m.	**Exhibit Hall Open** *Location: Grand Ballroom, 2nd Floor*
7:00 a.m. – 6:00 p.m.	**Registration** *Location: Columbus Ballroom Foyer, 2nd Floor*
8:00 a.m. – 9:00 a.m.	**WOMEN IN ANDROLOGY LECTURE** **Androgen Receptor Variation and Prostate Cancer in Humanized AR Mice** *Diane Robins, PhD* *University of Michigan* *(Introduced by Kate Loveland, PhD)*
9:00 a.m. – 9:15 a.m.	**Distinguished Service Award**
9:15 a.m. – 9:30 a.m.	**Quick Break**
9:30 a.m. – 11:00 a.m.	**SYMPOSIUM I – Meiosis in the Male** *Co-Chairs: Patricia L. Morris, PhD* *Petrice Brown, PhD* **Mismatch Repair Proteins in Male Meiosis** *Paula Cohen, PhD* *Cornell University* **Recombination, DNA Damage Repair and Meiosis** *Anton Grootegoed, PhD* *Rotterdam, The Netherlands* **MutS Homologues (hMSH4-hMSH5) Interactions During Meiosis** *Richard Fishel, PhD* *Ohio State University Medical Center*
11:00 a.m. – 12:30 p.m.	**Poster Session I** *Location: Grand Ballroom, 2nd Floor*
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** **"Shaping Our Research Communities: What Women Do Well and How to Do it Better!"** (Not included in registration fee; tickets required.) *Location: Riverview B, 3rd Floor* Joan M. Lakoski, PhD
2:00 p.m. – 3:30 p.m.	**CONCURRENT ORAL SESSIONS** **Oral Session I – Basic Research** *Location: Columbus Ballroom, 2nd Floor* Moderators: Barry Hinton, PhD Sophie-Ann Lamour, BSc

*Locations subjects to change

schedule at a glance

2:00 p.m. – 3:30 p.m.
Oral Session II – Clinical Research
Location: Washington Room, 3rd Floor
Moderators: Robert Oates, MD
Ajay Nangia, MBBS

3:30 p.m. – 3:45 p.m.
Refreshment Break
Location: Grand Ballroom, 2nd Floor

3:45 p.m. – 4:30 p.m.
LECTURE I
Prostate Stem Cells: Friend or Foe
Susan Kasper, PhD
Vanderbilt University Medical Center
(Introduced by Gail S. Prins, PhD)

4:30 p.m. – 5:15 p.m.
EAA LECTURE
The Y Chromosome Side of Male Fertility
Csilla Krausz, MD, PhD
University of Florence
(Introduced by Patricia Cuasnicu, PhD)

5:15 p.m. – 5:45 p.m.
CLINICAL LECTURE
Why Genetic Testing is Required in the Evaluation of the Severely Oligospermic / Azoospermic Male
Robert Oates, MD
Boston University
(Introduced by Dolores Lamb, 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: Washington Room, 3rd Floor

MONDAY, APRIL 6, 2009

7:00 a.m. – 12:30 p.m.
Exhibit Hall Open
Location: Grand Ballroom, 2nd Floor

7:00 a.m. – 6:00 p.m.
Registration
Location: Columbus Ballroom Foyer, 3rd Floor

8:00 a.m. – 9:00 a.m.
AUA LECTURE
Hypogonadism – Assessment of Quality of Life and New Tools for Clinical and Research Refinement
Raymond Rosen, PhD
New England Research Institutes
(Introduced by Dana A. Ohl, MD)

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

9:15 a.m. – 9:30 a.m.
Break

9:30 a.m. – 11:00 a.m.
SYMPOSIUM II – Regulation of Male Fertility: The Yin and the Yang
Co-Chairs: Janice P. Evans, PhD
Matthew Marcello, BS
Can a Safe, Effective, Reversible Male Contraceptive be Developed?
John Amory, MD
University of Washington

Properties of Spermatogonial Stem Cells
Dirk de Rooij, PhD
Utrecht University, The Netherlands

PLCzeta and Male Infertility
Rafael Fissore, PhD
University of Massachusetts

11:00 a.m. – 12:30 p.m.
Poster Session II
Location: Grand Ballroom, 2nd Floor

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 DIVERSITYAND TRAINEE AFFAIRS COMMITTEES
(Not included in registration fee; tickets required.)
Location: Riverview B, 3rd Floor
"Using PowerPoint Without PowerPoint Using You"
Barry T. Hinton, PhD
University of Virginia School of Medicine

12:30 p.m. – 2:00 p.m.
Editorial Board Luncheon
Location: Riverview A, 3rd Floor

2:00 p.m. – 3:00 p.m.
THE ASA INTERNATIONAL LECTURE
Homeobox Genes in Mammalian Sexual Development
Peter Koopman, PhD
University of Queensland
(Introduced by Patricia S. Cuasnicu, PhD)

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

schedule at a glance

3:30 p.m. – 5:00 p.m.

SYMPOSIUM III – Ejaculation and Ejaculatory Dysfunction
Co-Chairs: Peter Chan, MD
Darius A. Paduch, MD, PhD

Central and Peripheral Control of Ejaculation
Francois Giuliano, MD
University of Paris West

Premature Ejaculation-Epidemiology and Treatment
Wayne Hellstrom, MD
Tulane University Medical School

Current Trends in the Treatment of Infertility in Men with Spinal Cord Injury
Nancy Brackett, PhD
The Miami Project to Cure Paralysis

5:00 p.m.

ASA Business Meeting, Outstanding Trainee Investigator and Trainee Awards

7:00 p.m. – 11:00 p.m.

Annual Banquet – Independence Seaport Museum
(Not included in registration fee; tickets required.)
Location: Independence Seaport Museum

TUESDAY, APRIL 7, 2009

7:00 a.m. – 8:00 a.m.

2010 Program Committee Meeting
Location: USS New Jersey, 2nd Floor

7:30 a.m. – 12:15 p.m.

Registration
Location: Columbus Ballroom, 2nd Floor

8:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Advances in Andrology
Co-Chairs: David Karabinus, PhD
Joseph Alukal, MD

Single-Cell Analysis of Androgen Responses
Michael Mancini, PhD
Baylor College of Medicine

Essential Function of the First Bromodomain Motif in Bromodomain Protein for Spermiogenesis in Mice and Men
Debra J. Wolgemuth, PhD
College of Physicians and Surgeons of Columbia University

Microfluidic Applications in Andrology
Gary Smith, PhD
University of Michigan

9:30 a.m. – 10:00 a.m.

Refreshment Break
Location: Columbus Ballroom Foyer, 2nd Floor

10:00 a.m. – 10:45 a.m.

LECTURE II:
Male Circumcision and HIV/ST1 Infection Risk in Men and Women
Ronald H. Gray, PhD
Johns Hopkins University
(Introduced by Kirk C. Lo, MD, FRCSC)

10:45 a.m. – 12:15 p.m.

SYMPOSIUM V – Excurrent Genital Tract
Co-Chairs: Terry T. Turner, PhD
Joel Marmar, MD

Tubular Morphogenesis of the Developing Wolffian / Epididymal Duct: More Twists And Turns
Barry Hinton, PhD
University of Virginia School of Medicine

Amyloid in the Reproductive Tract: Pathology or Biological Function?
Gail Cornwall, PhD
Texas Tech School of Medicine

Post-Partum Initiation of Definition of Transcription in Various Epididymal Regions
John Herr, PhD
University of Virginia School of Medicine

MEETING ADJOURNED

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president's welcome

Wayne J.G. Hellstrom, MD, FACS

It is my pleasure and honor to welcome you to the 34th annual meeting of the American Society of Andrology (ASA) in the always-exciting city of Philadelphia.

We must realize that we are at an important place in time with much left to be done, as William Penn must have realized as he sat on the bank of the Delaware River envisioning a city in the wooded plains on either side of the river where now skyscrapers sprout from the ground and dominate the skyline. Penn named the unborn city Philadelphia after the Greek for "philos" love and "delphos" brother. Thus we convene, over 300 years later, in the thriving and beautiful "City of Brotherly Love" where we continue Penn's vision of progression by presenting the newest ideas in andrology to help our brothers love.

Our distinguished and worthy co-chairs, Dolores Lamb and Dana Ohl, have constructed a broadly focused and scientifically stimulating meeting aptly entitled "Bench to Bedside: Advances in Andrology." The prospectus includes a number of renowned keynote speakers who will deliver lectures and host symposia and related didactic sessions.

Dr. Arthur Burnett will once again host a stellar ASA Special Symposium on Saturday afternoon entitled "Issues in Urologic and Hormonal Health." This session will focus on major clinical and surgical themes in andrology. Due to its overwhelming popularity over the past number of years the Andrology Lab Workshop, chaired by Angela Reese, will be expanded to 1½ days (all Saturday and Sunday morning, April 4 – 5).

Our local hosts, Joel Marmar and Anelia Bollendorf, take pride in their city and assure us a wonderful time at the welcome reception and annual banquet.

I look forward to seeing you here in Philadelphia and hope you enjoy our outstanding 2009 ASA Annual Meeting.

Wayne J.G. Hellstrom, MD, FACS
President, ASA

Past Presidents of the American Society of Andrology

1975-1977	Emil Steinberger*	1993-1994	Bernard Robaire
1977-1978	Don W. Fawcett	1994-1995	Glenn R. Cunningham
1978-1979	C. Alvin Paulsen*	1995-1996	Marie-Claire Orgebin-Crist
1979-1980	Nancy J. Alexander	1996-1997	Arnold M. Belker
1980-1981	Philip Troen	1997-1998	Terry T. Turner
1981-1982	Richard M. Harrison	1998-1999	Richard V. Clark
1982-1983	Richard J. Sherins	1999-2000	Barry T. Hinton
1983-1984	Andrzej Bartke	2000-2001	J. Lisa Tenover
1984-1985	Rudi Ansbacher	2001-2002	Barry R. Zirkin
1985-1986	Anna Steinberger	2002-2003	Jon L. Pryor
1986-1987	William D. Odell	2003-2004	Gail S. Prins
1987-1988	Larry L. Ewing*	2004-2005	William J. Bremner
1988-1989	C. Wayne Bardin	2005-2006	Sally Perreault Damey
1989-1990	Rupert Amann	2006-2007	Christina Wang
1990-1991	Howard Nankin	2007-2008	Terry R. Brown
1991-1992	David W. Hamilton	*Deceased	
1992-1993	Ronald S. Swerdloff		

American Society of Andrology

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Treasurer	Erwin Goldberg, PhD
Past President	Terry R. Brown, PhD

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Ina Dobrinski, DVM, PhD; Michael K. Holland, PhD; Daniel S. Johnston, PhD
John P. Mulhall, MD; Charles H. Muller, PhD; Dana Alan Ohl, MD
Michael A. Palladino, PhD; Vassilios Papadopoulos, PhD; Carol S. Sloan, MS

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	Sally Perreault Damey, PhD; Research Triangle Park, NC (Co-Editor)
	Jay I. Sandlow, MD; Milwaukee, WI (Co-Editor)
Laboratory Science Forum	David S. Karabinus, PhD, HCLD; Fairfax, VA
Liaison Committee	Joel L. Marmar, MD; Philadelphia, PA
Local Arrangements Committee	Joel L. Marmar, MD; Philadelphia, PA
Membership Committee	Alan Diekman, PhD; Little Rock, AR (Co-Chair)
	Run Wang, MD; Houston, TX (Co-Chair)
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Program Committee	Dolores Lamb, PhD; Houston, TX (Co-Chair)
	Dana Alan Ohl, MD; Ann Arbor, MI (Co-Chair)
Public Affairs and Policy Committee	Moshe Wald, MD; Iowa City, IA
Publications Committee	Marvin L. Meistrich, PhD; Houston, TX
Testis Workshop	E. Mitch Eddy, PhD; Research Triangle Park, NC
Trainee Affairs	Michael A. Palladino, PhD; West Long Branch, NJ

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NOTICE TO READERS

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

general information

About Philadelphia, Pennsylvania

The City of Brotherly Love is home to world-class museums, artistic venues, a variety of culinary options, tax-free shopping, and historic landmarks, such as the Betsy Ross House, Valley Forge National Historic Park, and of course, the Liberty Bell and Declaration of Independence.

Shopping
Enjoy a wonderful mix of urban shopping districts, some of the nation's largest retail centers and tax-free shopping on clothing and shoes. Visit Center City, where you can find one-of-a-kind boutiques and independent shops offering handcrafted, unique and quirky gifts, as well as some nationally known retailers. The biggest names in shopping can be found along Rittenhouse Row, with Urban Outfitters, Polo Ralph Lauren, J. Crew, Williams-Sonoma and Diesel to name a few. You can also visit the largest urban mall in America at The Gallery at Market East, Jewelers' Row, the country's oldest diamond district, and Antique Row, a shopping area full of exquisite treasures and expert advice. For a more eclectic selection, head to University Square, Main Street in Manayunk, or the cobblestone streets of Chestnut Hill. Dozens of charming main streets overflowing with Americana and shopping treasures are a short drive from Center City in towns such as West Chester, Media and New Hope. Also a short ride from Center City is the largest retail complex in the country, The King of Prussia Mall. For great deals, check out the Franklin Mills Mall, the official landmark of the discount shopper, and its 220 brand name discount stores.

Dining
Philadelphia may be most famous for cheese steak, but is also one of our country's finest culinary destinations. Throughout the city, four-star dining rooms coexist with unassuming neighborhood bistros; upscale taprooms, and family-operated bring-your-own-bottle (BYOB) establishments share the sidewalk with high-profile restaurants owned by star restaurateurs. Standout restaurants like Lacroix, Vetri, Le Bec-Fin, Alma de Cuba and the White Dog Café lead the way. *Saveur* magazine named Philadelphia the nation's "most underappreciated American food town" so be sure to check out some of these amazing places to dine while you're in town.

Historic Attractions
Even before William Penn founded the city and built his estate in Bucks County, Native Americans and Swedish colonists recognized the region as an attractive place for trade and settlement. Enjoy the legacy from more than three centuries of political, economic, social, and cultural achievements — all within the Philadelphia area.

It's just one square mile, but the area between Front to 7th Streets and Spruce to Race Streets covers three different neighborhoods, all jam packed with restaurants, galleries, shops and attractions. Each puts its own distinctive spin on history. And together, they make up Historic Philadelphia. There, America's most sacred historic sites (the Liberty Bell and Independence Hall) stand side-by-side among the hustle and bustle of a modern city. Old City brims with shops, cafes, restaurants and bars while Society Hill welcome visitors to stroll their cobblestone streets and take in the architecture of the centuries-old homes, churches and landmarks that make the neighborhood so picturesque.

Museums and the Arts
There are several different types of museums in the Philadelphia area. On a grand scale, is The Franklin Institute Science Museum and more intimate is the Polish American Cultural Center Museum. Also there are the more specialized museums like The Philadelphia Insectarium and the Philadelphia Athletics Museum. Philadelphia is a city of firsts, and it holds a first in the art category too: the Pennsylvania Academy of the Fine Arts is the oldest art museum in the country, founded in 1805 by Charles Willson Peale, a painter himself who also fathered distinguished artists, Rembrandt and Raphaelle Peale.

Zoos and Aquariums
The Philadelphia Zoo is the oldest zoo in the United States, celebrating 150 years on March 21, 2009. The 42-acre campus, featuring a variety of habitats, gives you an up close and personal experience with animals from around the world. Train and boat rides, horseback tours and camel safaris bring you right in the middle of the action for an insider's perspective of the wildlife.

New to the area is the Adventure Aquarium where you can explore nearly 200,000 square feet of sea life and wildlife at a brand new, state-of-the-art riverfront aquarium. Visit a West African River featuring hippopotamuses, crocodiles, porcupines and over 20 species of African birds in a free-flight aviary; surround yourself with sharks in a suspended 40-foot walk-through tunnel; experience the wonders of the deep sea in the Jules Verne Gallery; and you can even have an once-in-a-lifetime opportunity to "Swim with the Sharks."

Weather
In April, the temperature in Philadelphia ranges from an average low of 44° F to an average high of 62° F.

Hotel Information

Hyatt Regency Philadelphia at Penn's Landing
201 S. Columbus Blvd.
Philadelphia, PA 19106
Phone: (215) 928-1234
Fax: (215) 521-6600
www.Pennslanding.hyatt.com

Hotel Accommodations
$199.00 single/double/triple/quad, guest room tax is currently 14% (subject to change). All reservations must be made individually through the hotel's reservation department by calling (215) 928-1234 or (800) 233-1234. Or you may visit the website at www.pennslanding.hyatt.com and input the Group Code: G-ANDR.

Guests will need to provide a form of payment (credit card) to guarantee the reservation at the time of making the reservation. Note that check-in is at 3:00 p.m. and checkout is at 12:00 p.m. Upgrades to river view rooms, business plan and executive king rooms are available for an additional $25.00 per room for each occupancy type per night.

Transportation

Philadelphia International Airport (PHL)

Car Rental
Avis Rent-A-Car is the official rental car company for the ASA 2009 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 or Shuttle Service
Taxi: $28.50

Lady Liberty: $11 per person. Proceed to a phone in baggage claim area and dial 27 for pickup for ground transportation. The van makes multiple stops and runs every 20 minutes from 5:30 a.m. to midnight. For more information, call (215) 724-8888.

Laboratory Science Forum Luncheon
"Leukocytes and Spermatocytes: Seminal Clues to Reproductive Health"
Date: Saturday, April 4, 2009
Time: 11:45 p.m. – 1:15 p.m.
Location: Riverview C, Third Floor
Join us for the 2009 Lab Science Forum Luncheon. The luncheon will include a talk entitled "Leukocytes and Spermatocytes: Seminal Clues to Reproductive Health", addressing non-sperm components of semen and how they can be used as a diagnostic tool for evaluating male reproductive health, given by Charles H Muller, PhD, HCLD, from the University of Washington School of Medicine. The luncheon will take place during the lunch break of the Andrology Laboratory Workshop (ALW) and is included in the ALW registration fee.
Cost: $35.00 for non-ALW registrations. Visit the registration desk for more information.

Welcome Reception
Date: Saturday, April 4, 2009
Time: 7:45 p.m. – 9:30 p.m.
Location: Grand Ballroom, Second Floor
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 Testis Workshop, Special Symposium and/or Andrology Lab Workshop.
Dress: Business casual or casual attire is appropriate

Women in Andrology Luncheon and Discussion
"Shaping Our Research Communities: What Women Do Well and How to Do it Better!"
Date: Sunday, April 5, 2009
Time: 12:30 p.m. – 2:00 p.m.
Location: Riverview C, Third Floor
Joan M. Lakoski, PhD, is the associate vice chancellor for Academic Career Development, founding and executive director of the Office of Academic Career Development, professor, Clinical and Translational Science Institute at the University of Pittsburgh Health Sciences, and associate dean for postdoctoral education and professor of pharmacology and chemical biology at the University of Pittsburgh School of Medicine. Her administrative responsibilities encompass oversight and development of comprehensive career development services, including mentoring programs for professional students, postdoctoral fellows, residents, clinical fellows and faculty across the health schools at the University of Pittsburgh. Her talk will address strategies to achieve our research goals, consider practical approaches to achieve positive outcomes and effective collaborations, and sustain career satisfaction and success as a women scientist.
Cost: $25.00 per person. Visit the registration desk for more information.

Trainee Forum and Mixer
Date: Sunday, April 5, 2009
Time: 6:30 p.m. – 8:30 p.m.
Location: Washington Room, Third Floor
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. Visit the registration desk for more information.

Mentoring Luncheon Sponsored by the Diversity and Trainee Affairs Committees
"Using PowerPoint Without PowerPoint Using You"
Date: Monday, April 6, 2009
Location: Riverview B, Third Floor
Time: 12:30 p.m. – 2:00 p.m.
In this session we will explore how slideware such as PowerPoint and Keynote can be used with imagination, creativity and innovation to keep your audience — whether students or colleagues — fully engaged. Bullet-point-laden slides seem to be norm, but is this the most effective way of getting your points across? We will examine ways to improve the design of your slides in order to tell a story that promotes learning and engagement. In addition, we will discuss the virtues and vices of presentation software: When is PowerPoint appropriate for your presentation and when does it promote "Death by PowerPoint"? Be prepared to be an active participant in the discussions with speaker, Barry T. Hinton, PhD, Professor of Cell Biology at the University of Virginia School of Medicine.
Cost: $5.00 for trainees, $10.00 for non-trainees. Visit the registration desk for more information.

Annual Banquet
Date: Monday, April 6, 2009
Time: 7:00 p.m. – 10:00 p.m.
Location: Independence Seaport Museum (located next to the hotel)
Join ASA for a flashback to the 1950s and *AMERICAN BANDSTAND*! Enjoy the sounds of Jerry Blavet, one of the original dancers on the show, as he plays the sounds and songs of the 50s. Ladies, come dressed in your taffeta dress or poodle skirt and guys, do not forget your Pat Boone white bucks, leather coat and jeans with your hair slicked back to dance the night away like you were on AMERICAN BANDSTAND!
Cost: $75.00 per person, $35.00 for trainees. Includes dinner and entertainment. Please sign up for this event on the registration form
Dress: Taffeta dress/poodle skirt for the ladies and white bucks, leather coat and jeans for the guys (casual)

Program Committee
Dolores Lamb, PhD; Houston, TX (Co-Chair)
Dana Alan Ohl, MD; Ann Arbor, MI (Co-Chair)
Ashok Agarwal, PhD; Cleveland, OH
Arthur L. Burnett, II, MD; Baltimore, MD
Peter Chan, MD; Montreal, PQ, Canada
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Janice P. Evans, PhD; Baltimore, MD
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Antoine A. Makhlouf, MD, PhD; Minneapolis, MN
Joel L. Marmar, MD; Philadelphia, PA
Patricia Morris, PhD; New York, NY
Robert D. Oates, MD; Boston, MA
Darius A. Paduch, MD, PhD; Red Bank, NJ
Michael A. Palladino, PhD; West Long Branch, NJ
Gail S. Prins, PhD; Chicago, IL
Jay I. Sandlow, MD; Milwaukee, WI
Rebecca Z. Sokol, MD, MPH; Los Angeles, CA
Paul Jacob Turek, MD; San Francisco, CA

message from the program co-chairs

Dolores Lamb, PhD and Dana Ohl, MD

On behalf of the 2009 Program Committee, we welcome you to Philadelphia and the 34rd Annual Meeting of the American Society of Andrology (ASA). The theme of this year's meeting is "Bench to Bedside: Advances in Andrology" with basic, translational and clinical lectures focusing on cell and molecular biology, urology, endocrinology and men's health. A broad range of topics will be presented beginning Saturday night with a lecture by Dr. Blanche Capel, presenting the ASA Keynote Lecture. Dr. Capel is a professor in the Department of Cell Biology at Duke University Medical School. Her studies use a variety of approaches such as organ culture, transgenic mice, confocal microscopy and biochemical and molecular approaches to define the cascade of events controlling sex determination. The Women in Andrology Lecture entitled "Androgen Receptor Variation and Prostate Cancer in Humanized AR Mice" will be given by Diane Robins, PhD, from the University of Michigan School of Medicine. Dr. Robins has extensively studied the mechanism of androgen action, most recently focusing on the development of a novel mouse model for prostate cancer. The ASA International Lecture will be given by Peter Koopman, PhD on "Homeobox Genes in Mammalian Development". Csilla Krausz, MD, the EAA Lecturer will lecture on "The Y Chromosome Side of Male Fertility" followed by Robert Oates, MD speaking on "Why Genetic Testings is Required in the Evaluation of the Severely Oligospermic/Azoospermic Male".

All aspects of male reproduction will be included with the AUA lecture focused on hypogonadism (Raymond Rosen, MD). Prostate stem cells (Susan Kasper, PhD) and male circumcision (Ronald H. Gray, PhD) lectures complement symposiums on the excurrent genital tract, advances in andrology, ejaculation and erection, contraception and meiosis. Andrology research has rapidly advanced over the past year with new concepts and technologies developed. Indeed, the meeting has been designed to explore new advances that will drive basic and clinical research in the future, ultimately impacting patient diagnosis and treatment.

Our goal for this meeting is to present exciting advances in diverse research areas in our broad field of andrology. We hope that these lectures will broaden and enhance your research and/or clinical practice. The American Society of Andrology is a relatively small, but highly collegial group. If you are not currently a member, we encourage you to join the society. The opportunity for informal scientific discussions and social interactions among the attendees represents a definite strength of the Society.

The members of the 2008 Program Committee deserve a great deal of credit for the selection of speakers for this annual meeting. The members include: Wayne Hellstrom, MD, the current ASA President, Arthur Burnett, MD, Paty Cuasnicu, PhD, Gail Prins, PhD, Janice Evans, PhD, Patricia Morris, PhD, Tracy Rankin, PhD, Michael Palladino, PhD, Joel Marmar, MD, Kirk Lo, MD, Kate Loveland, PhD, Ashok Agarwal, PhD, Robert Brannigan, MD, Stanton Honig, MD, Laurence Levine, MD, Robert Oates, MD, Jay Sandlow, MD, Steven Schrader, PhD and Gary Smith PhD. We also thank Gail Cornwall, PhD, Paul Turek, MD.(2008 Program Chairs) and Mitch Eddy, PhD (Testis Workshop 2009 Program Chair) for their invaluable guidance and all members of the ASA who suggested program topics and speakers.

This year the Program Committee has worked to develop a program that encompasses all areas of men's reproductive health reflecting the Society's diversity and its members' interests. Leading experts in sex determination and development, steroid hormone action, meiosis, prostate stem cells, male infertility, ejaculation, sexually transmitted infectious diseases, sexual medicine, andrology and genital tract biology will present.

An important non-overlapping, CME-accredited, clinical satellite symposium, chaired by Arthur Burnett, MD, Johns Hopkins University, will occur the day before the annual meeting. This symposium is entitled "Issues in Urologic and Hormonal Health". The beginning lectures start with a focus on androgens. Lisa Tenover, MD, PhD will speak on "Testosterone and Bone Health", followed by Andrew McCullough, MD presenting the newest updates on "Testosterone and Other Therapies for Penile Health After Radical Prostatectomy". The advantages and possible caveats of androgen replacement in men with respect to prostate cancer will be the focus of Abraham Morgentaler, MD's presentation on "5-Alpha Reductase Inhibitor Effects on Prostate and Sexual Function" will be discussed by Stephanie Page, MD, PhD. Later in the afternoon, the focus will shift to BPH/LUTS and erectile dysfunction (Ajay Nehra, MD) and therapies for Peyronie's Disease (Wayne Hellstrom, MD). The symposium is of broad interest to urologists and endocrinologists, as well as the basic researchers and promises to include provocative discussions of these sometimes controversial, but also critical issues for andrologists.

The 2009 ASA Andrology Laboratory Workshop and Laboratory Science Forum will also be held the day before the annual meeting. The Andrology Laboratory Workshop program is entitled "2009 Andrology Laboratory Workshop with a Novel Morphology Consensus Workshop," and features ASA members Aniela Bollendorf, MT, David Karabinus, PhD, HCLD, Dean Morbeck, PhD, Angela Reese, TS, Susan Rothmann, PhD, HCLD, Steven Schrader, PhD and Steve Simon, PhD. The Laboratory Science Forum Luncheon will include a lecture by Dr. Charles Muller entitled "Leukocytes and Spermatocytes: Seminal Clues to Reproductive Health".

Awards will be presented that recognized the contributions of our members to the field and to our society. Successful new investigators, as well as distinguished researchers in andrology, will be given during the meeting. The Distinguished Andrologist Award is presented annually to an individual who has made an outstanding contribution to the field of andrology. The Distinguished Service Award is bestowed each year to recognize those individuals who have played many important roles in the ASA. Several trainee awards will also be given at the meeting and we strongly encourage you to support our future leaders in andrology.

In addition we wish to acknowledge the continuing support of this 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 & Associates, especially Debbie Roller, Ann Marie Bray, Ruth Gottmann, Donna Kelly, and Marc Cakanic for their assistance in all aspects of creating and executing this meeting.

Looking forward to seeing you in Philadelphial

Dolores J. Lamb, PhD, HCLD
Dana Ohl, MD
2009 Program Co-Chairs

asa lecturer award

Blanche Capel, PhD

Blanche Capel, PhD, is a professor in the Department of Cell Biology at Duke University Medical Center. Dr. Capel's work is focused on the molecular control of mammalian gonad development. She received her BA degree in literature and art history from Hollins College and then attended Haverford College for additional studies in molecular biology and genetics. She trained with Dr. Beatrice Mintz and received her PhD from the University of Pennsylvania in genetics. Following post-doctoral training at the NIMR with Dr. Robin Lovell-Badge, Dr. Capel joined the faculty at Duke University Medical Center in 1993, where she rose to the rank of professor. She has been honored with the Gordon G. Hammes Faculty Teaching Award at Duke (2006), the Neena B. Schwatz Lectureship in Reproductive Biology (2007) and the Langford Lecture (1999). Dr. Capel has also chaired or co-organized important meetings in the areas of molecular embryology of the mouse (2003 - 2006), germ cell genetics (2002 - 2004; Cold Spring Harbor) and organized the Fourth International Symposium on Vertebrate Sex Determination (2006). She serves on the Board of Scientific Overseers for the Jackson Laboratory and on a number of committees at Duke University and the Medical Center.

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

distinguished andrologist award

William Bremner, MD, PhD

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. William Bremner of the University of Washington as the 2009 Distinguished Andrologist for his outstanding contributions in the realm of reproductive endocrinology and his exceptional legacy of prominent andrologists that he has mentored throughout his career.

Dr Bremner graduated from Harvard University and received his medical degree from the University of Washington School of Medicine in 1969. Following a residency in internal medicine and a fellowship in endocrinolgoy at the University of Washington, he went on to work with David DeKrester at Monash University in Melbourne, Australia. There, he not only earned his PhD in physiology and medicine, but also served on the Australian National Health and Medical Research Council as Senior Research Officer and Honorary Physician in Endocrinology and Diabetes. He returned to the University of Washington in 1977 and became chief of endocrinology and then chief of medicine at the Veterans' Affairs Puget Sound Health Care System. In 1998, he became chair of the Department of Medicine at the University of Washington.

Dr Bremner is best known for his research in reproductive endocrinology. He performed groundbreaking work on the relative roles of LH and FSH in controlling testicular function and helped to establish the relative roles of testosterone, dihydrotestosterone, estradiol and Inhibin B on gonadotropin secretion. He also investigated the physiological effect of androgens in men and the effects of aging on the reproductive system. In addition, he has performed seminal work on the development of an effective reversible hormonal contraceptive for men. In support of this research, he co-founded and then directed the Population Center for Research in Reproduction at the University of Washington until 2004. He has also served on the Steering Committee for the World Health Organization Special Programme on Research in Human Reproduction.

Dr Bremner's contribution to the field of andrology went beyond research. His direct involvement in the ASA has been enormous. In 2004, he served as president of the society, and prior to that served numerous other roles such as council member and program chair of the 1999 ASA meeting. His unique enthusiasm and optimism has allowed him to attract and encourage medical residents and endocrinology fellows to enter the field of andrology. The many trainees he mentored at various times include prominent researchers in the field — too many to list here — most of whom continue to be active in academic andrology and research in the US and overseas. Some have in fact been recognized by the ASA as outstanding researchers in their own right. His continued commitment to training the next generation of andrologists is evidenced by his current support and mentorship of new andrology fellows at the University of Washington.

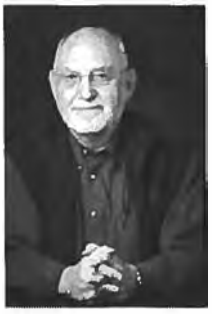
Dr Bremner's achievements and service have garnered him many awards. He is a member of the Alpha Omega Alpha Medical Honorary Society, and a recipient of the Henry Christian Award for Excellence in Research from the American Federation for Clinical Research. He has also been elected to both the American Society of Clinical Investigation and the Association of American Physicians honorary societies, and is the immediate past president of the Association of Professors of Medicine. In 2003 he received the University of Washington School of Medicine Distinguished Alumni Award and in 2005 he was given the Mayo Soley Award by the Western Society of Clinical Investigation in recognition of lifetime achievement in scientific endeavors and for his concern for junior faculty. In recognition of Dr Bremner's many accomplishments, the ASA is proud to add to that list by selecting him as our Distinguished Andrologist for 2009.

Distinguished Andrologists

1976	Roy O. Greep & M.C. Chang	1993	Anna Steinberger
1977	Robert E. Mancini	1994	Richard J. Sherins
1978	Robert S. Hotchkiss	1995	Rupert P. Amann
1979	Thaddeus Mann	1996	J. Michael Bedford
1980	John MacLeod	1997	Brian P. Setchell
1981	Alexander Albert	1998	Ryuzo Yanagimachi
1982	Eugenia Rosemberg	1999	Richard D. Amelar
1983	Kristen B.D. Eik-Nes	2000	Bayard T. Storey
1984	Mortimer B. Lipsett	2001	Frank S. French
1985	Robert H. Foote	2002	Geoffrey M. H. Waites
1986	Alfred D. Jost	2003	David M. de Kretser
1987	Emil Steinberger	2004	Ronald Swerdloff
1988	Yves W. Clermont	2005	Mitch Eddy
1989	C. Alvin Paulsen	2006	Norman Hecht
1990	Marie-Claire Orgebin-Crist	2007	Eberhard (Ebo) Nieschlag
1991	Philip Troen	2008	Bernard Robaire
1992	C. Wayne Bardin		

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

distinguished service award



Erwin Goldberg, PhD

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. Erwin Goldberg, professor of biochemistry, molecular biology and cell biology at Northwestern University, Illinois. A native of New York State, Dr. Goldberg received his PhD from the University of Iowa in 1956. He went on to complete his post-doctoral training at Iowa. In 1963, he joined Northwestern University, where he has been extremely productive in researching spermatogenesis, with over 180 publications deriving from his work.

Dr. Goldberg has been a member of the ASA since it was founded in 1975, and his devotion to the society has continued undiminished since then. He has quietly and effectively served the ASA in a wide range of capacities. He served on multiple committees, including the Annual Meeting Program Committee (1994 – 1996, 1999 and as chair in 2002), Awards Commitee (1991 – 1994), Future Meetings Committee (1979 – 1981 and 2003 – 2008), Post-Graduate Course Program Committee (1999) and the Local Site Committee as chair in 2003. He also served the Executive Committee of the North American Testis Workshop from its inception till the present and served as program chair for the workshop in 1999. When the Testis Workshop became formally aligned with the ASA in 2002, Dr. Goldberg served as a liaison between the two organizations and ensured the success of this union. In 2002, Dr. Goldberg directed the program for the ASA Annual Meeting in Seattle which broke the meeting attendance record at the time. He currently serves as Treasurer (2008 – 2011) and member of the *Journal of Andrology* Editorial Board.

The breadth of Dr. Goldberg's service to the ASA is all the more remarkable for having been performed quietly, effectively and without fanfare. His uncanny ability in negotiating venues for meetings resulted in significant fiscal savings for the society at a time of financial difficulty. His encouragement of ASA meeting attendance by his many trainees and graduates over the years has strengthened the membership base. And his long history and expertise with the society continues to be the source of wise advice at council meetings.

The ASA is proud to recognize Dr. Goldberg's 34 years of dedicated service by gratefully presenting him with the Distinguished Service Award for 2009.

Distinguished Service Award Recipients

1994	C. Alvin Paulsen
1995	Andrzej Bartke
1996	Philip Troen
1997	Marie-Claire Orgebin-Crist
1998	Rupert P. Amann
1999	David W. Hamilton
2000	Bernard Robaire
2001	Gail S. Prins
2002	Terry T. Turner
2003	Arnold M. Belker
2004	J. Lise Tenover
2005	Barry Hinton
2006	Barry Zirkin
2007	Sally Perreault Darney
2008	Matthew P. Hardy

young andrologist award

Michael A. Palladino, PhD

The Young Andrologist Award recognizes the contributions to the field of andrology by a member of the American Society of Andrology under 45 years of age. Dr. Michael A. Palladino of Monmouth University is this year's recipient. Dr Palladino obtained his undergraduate degree from Trenton State College (now known as The College of New Jersey) in New Jersey in 1987. He received his PhD degree from the University of Virginia in Charlottesville in 1994 under the mentorship of Barry Hinton. In 1999 he joined the faculty in the Department of Biology at Monmouth University, New Jersey. In 2004, he was promoted to associate professor and in July 2008 was appointed dean of the School of Science, Technology and Engineering. Despite his new duties as dean, Dr Palladino continues to be active in teaching undergraduates and running a successful basic science research laboratory.

Dr Palladino's research has focused on the protection of spermatozoa and cells of the male excurrent tract from the insults of foreign microorganisms and reactive oxygen species, and molecular mechanisms of testicular torsion injury. His graduate and post-doctoral work demonstrated the regulation of g-glutamyl transpeptidase expression, a key component of the anti-oxidative machinery of the epididymis, by androgens and testis-specific luminal factors. In later work, he examined the expression of the anti-microbial defensin proteins in the epididymis. His laboratory is currently studying antimicrobial signaling in the epididymis via Toll-like receptor (TLR) pathways, and roles of the transcription factor hypoxia-inducible factor-1 in oxygen homeostasis in the testis and the protection of Leydig cells from apoptosis following testis ischemia. Despite working at a primarily undergraduate institution, Dr. Palladino has been very successful in securing NIH funding support for work. He has also received funding from a number of other federal and private sources. His research productivity is evidenced by authorship or co-authorship of over 30-peer reviewed publications, many in the top reproductive journals. In addition, Dr Palladino is a premier educator. At Monmouth, he has trained over 60 undergraduate students in his laboratory, many of whom have presented at national and international conferences, received awards for their research, and have completed graduate and medical programs. He has taught numerous courses in disciplines such as biotechnology, genetics, endocrinology, and cell and molecular biology. Dr. Palladino has developed and written several curricula and undergraduate textbooks. He is the co-author *of Introduction to Biotechnology*, the leading undergraduate textbook in the field that is used at over 100 institutions domestically and internationally, co-author of *Concepts of Genetics*, 9e and *Essentials of Genetics*, 7e two premier textbooks in the field, and author and editor for the *Special Topics in Biology* series. Collectively these publications have helped educate over 500,000 students worldwide.

Closer to home, he has been very supportive of ASA trainees through his outstanding work on the Trainee Affairs Committee for the past 7 years including the past 3 years as committee chair and he served as advisor for the SSR Trainee Affairs Committee from 2001 – 2008. He is currently a member of the ASA Executive Council, the 2009 and 2010 Conference Program Committees, and the *Journal of Andrology* Editorial Board. Dr. Palladino's past service to ASA includes the Andrology Laboratory Workshop Committee (2003 – 2006, chair 2004 – 2005), Minority Affairs Committee, and the Development Committee. His efforts has been recognized by numerous awards including the 2005 Distinguished Teacher Award from Monmouth University, the 2005 Caring Heart Award from the New Jersey Association for Biomedical Research, the 1997 – 1998 Outstanding Colleague Award for teaching excellence from Brookdale Community College, and the ASA New Investigator Award in 1993.

In summary, Dr Palladino has been very successful in balancing education with research. His remarkable rise at his institution is testimony to his skill and perseverance. The ASA is proud to recognize him as this year's recipient of the Young Andrologist Award.

Young Andrologist Award Recipients

1982	L.J.D. Zaneveld	1996	Paul S. Cooke
1983	William B. Neaves	1997	Gail A. Cornwall
1984	Lonnie D. Russell	1998	William R. Kelce
1985	Bruce D. Schanbacher	1999	Stuart E. Ravnik
1986	Stephen J. Winters	2000	Matthew P. Hardy
1987	Ilpo T. Huhtaniemi	2001	Jacquetta Trasler
1988	Larry Johnson	2002	Christopher L.R. Barratt
1989	Barry T. Hinton	2003	Joanna E. Ellington
1990	Luis Rodriguez/Rigau	2004	Kate Loveland
1991	Patricia M. Saling	2005	Janice Bailey
1992	Gary R. Klinefelter	2006	Janice P. Evans
1993	Robert Chapin	2007	John K. Amory
1994	Wayne J.G. Hellstrom	2008	Moira K. O'Bryan
1995	Christopher DeJonge		

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

outstanding trainee investigator award

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

The recipient of the 2009 New Investigator Award will be announced during the Annual Business Meeting on Monday, April 6, 2009 at 5:00 p.m.

New Investigator Award Recipients

1983	Thomas T. Tarter	1996	Wei Gu, Daniel B. Rudolph
1984	Peter S. Albertson	1997	Loren D. Walensky
1985	Randall S. Zane	1998	Dolores D. Mruk
1986	Mark A. Hadley	1999	Jacques J. Tremblay
1987	Peter Grosser	2000	Jeffrey J. Lysiak
1988	Stuart E. Ravnik	2001	Alexander T.H. Wu
1989	Tracy L. Rankin	2002	Ebtesam Attaya
1990	Donna O. Bunch	2003	Mustafa Faruk Usta
1991	Robert Viger		
1992	John Kirby		
1993	Michael A. Palladino		
1994	Linda R. Johnson		
1995	Mehdi A. Akhondi		

Outstanding Trainee Investigator Award Recipients

2004	Darius Paduch
2005	Tara Barton
2006	Liwei Huang
2007	Steve Tardif
2008	Duangporn Jamsai

thanks to donors, supporters and exhibitors

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

Gold Level
(Multiple or single contribution(s) greater than or equal to $10,000)
James Nelson, III, MD
Eugenia Rosemberg, MD
Richard Sherins, MD
Texas Institute for Reproductive Medicine and Endocrinology
West Michigan Reproductive Institute

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

Sustaining
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Arnold M. Belker, MD
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E. Mitch Eddy, PhD
Erwin Goldberg, PhD
Glenn R. Cunningham, MD
Marc Goldstein, MD
Richard D. Amelar, MD
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Richard Van Clark, MD, PhD
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Rudi Ansbacher, MD
Rupert P. Amann, PhD
Terry T. Turner, PhD
Wayne J.G. Hellstrom, MD
William J. Bremner, MD, PhD

Annual Contributions for Fiscal 2008

1000+
William J. Bremner, MD, PhD
Douglas T. Carrell, PhD
Wayne J.G. Hellstrom, MD
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Bernard Robaire, PhD
Richard J. Sherins, MD
Ronald Swerdloff, MD
Donna L. Vogel, PhD
Christina Wang, MD

$250 – $999
Rupert P. Amann, PhD
Barry T. Hinton, PhD
Joel L. Marmar, MD
Jon Lee Pryor, MD
J. Lisa Tenover, MD, PhD
Terry T. Turner, PhD

$100 – $249
John K. Amory, MD
Janice P. Evans, PhD
Frank S. French, MD
George L. Gerton, PhD
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Sami A. Hamdi, MD
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Stuart E. Ravnik, PhD
Rebecca Z. Sokol, MD, MPH
Bayard T. Storey, PhD
Jan Karol Wolski, MD

$50 – $99
Andrzej Bartke, PhD
Arthur L. Burnett, II, MD
Martine Culty, PhD
Christopher J. De Jonge, PhD, HCLD
Kate Loveland, PhD
Sally Perreault Damey, PhD
Barbara M. Sanborn, PhD
Hitoshi Takeshima, MD
Nancy E. Warner, MD

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.*

course objectives & CME credit information

Andrology Lab Workshop 2009

"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 2009 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. After the presentation, each participant will receive virtual smears with photographed sperm images to perform a morphology assessment. Faculty will be available to help as needed. Participants may keep the virtual smears for future reference and practice.

In the afternoon, data from analyzing the 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. Clinical case studies will be discussed as well, to provide a link between what the technologist is seeing on the smears and what the physician is looking for on the reports.

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

ASA Satellite Symposium 2009

"Issues in Urologic and Hormonal Health"

Statement of Needs

Concern for male health applies to numerous clinical conditions related to urologic and hormonal function. Common disorders in men relate to androgen deficiency, and these both influence and are influenced by metabolic disorders, cardiovascular disease, prostate disease, and sexual dysfunction. Management of these disorders may include androgen treatment with effects on multiple target organs. It is important to understand the extent of health problems which occur in men and the implications of various urologic and hormonal treatments for the preservation of male health.

Learning Objectives

At the conclusion of the Satellite Symposium, participants should be able to:

- Identify the health problems associated with androgen deficiency in men
- Review the beneficial effects and risks associated with testosterone treatment in the men with androgen deficiency
- Describe the role of various treatments for urologic and hormonal conditions, including those related to prostate and sexual health, as these relate to overall preservation of male health

34th Annual ASA Meeting

"Bench to Bedside: Advances in Andrology"

Statement of Needs

Advances in our understanding of the molecular controls of male reproductive function have lead to improved understanding of male reproductive defects seen by the clinician. Indeed, there has been an exponential increase in our understanding of the basic mechanisms regulating every aspect of sex determination, differentiation, testicular and genital tract development and function; this knowledge will ultimately improve patient diagnosis and treatment. In addition, the improved knowledge of the controls of erection and ejaculation are increasingly leading to novel therapies of conditions such as ejaculatory failure/dysfunction and erectile dysfunction. Advances in the clinical andrology laboratory procedures used to diagnose and treat the infertile couple have improved outcomes and streamlined practice. The translation of the basic and translational research findings to effect clinical evaluation and treatment requires the effective communication and interaction between basic and clinical andrologists to mutually learn the problems faced by the clinicians and the scientific advances that may impact these clinical challenges.

This program will provide essential information about emerging concepts and technologies that are being used to advance knowledge in male reproductive health disorders. It will offer professionals in andrology, including male health specialists, urologist, gynecologists, reproductive endocrinologists, biochemists, geneticists, molecular, cell and developmental biologists and laboratory technicians, the opportunity to increase and integrate their knowledge of the technological and methodologic advances occurring in andrology. Finally, it will also help clinicians incorporate these concepts and technologies into everyday clinic practice.

Learning Objectives

At the conclusion of this program, attendees should be able to:

- Describe the advances altering our understanding of sex determination and differentiated function
- Recognize the important molecular controls now recognized in meiosis during spermatogenesis
- Explain how prostate stem cells are regulated, proliferated and differentiated and how they may play a role in prostate cancer
- Describe the current understanding of the genetics of male infertility and the role of genetic testing
- Explain the new technological advances in Andrology with regard to androgen action and clinical Andrology diagnosis and treatment
- Review how the erection and ejaculation occurs, and identify the key defects that contribute to ejaculatory defects
- Describe the development and function of the excurrent genital tract in the male
- Assess the past and current issues in the study of male circumcision, the risk of HIV/ST1 infection and how epidemiologic investigations can forge new basic science research avenues

XX North American Testis Workshop

"Testicular Function: Levels of Regulation"

The theme of the 2009 workshop is "Testicular Function: Levels of Regulation." The previous workshop marked the transition from the genomic to the post-genomic era. With the decoding of linear genome sequences nearing completion, it recognized that a foundation was established on which to construct an understanding of the genetic programs, epigenetic influences, and signaling events that regulate gene expression at the chromosome and transcript levels. This 2009 workshop extends this theme by emphasizing that regulation of the spermatogenic and endocrine functions of the testis are highly interactive processes that occur at multiple levels. It explores how this happens through intrinsic genetic programs, interactions between germ cells and somatic cells, and extrinsic signals that influence these intrinsic and interactive processes. The different levels of regulation are represented in the five plenary sessions on Testis Development, Testis Function, Germ Cell Development, Gene Expression in Germ Cells, and Gamete Development and Function.

Learning Objectives

At the completion of this meeting, participants should be able to:

- Recognize the major features of testicular function and dysfunction in mammals
- Distinguish the cellular mechanisms and signaling pathways involved in testis development
- Identify the role of small RNAs and epigenetic processes in regulating germ cell line gene expression
- Describe the endocrine pathways and components essential for testicular function
- Assess the role of evolution and genome divergence in determining male fertility

CME credit information

Accreditation Statement

This activity has been planned and implemented in accordance with the

Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of Oklahoma College of Medicine and American Society of Andrology. The University of Oklahoma College of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

The University of Oklahoma College of Medicine designates this educational activity for a maximum of **26.25** *AMA PRA Category 1 Credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Conflict Resolution Statement

The University of Oklahoma College of Medicine, Office of Continuing Medical Education has reviewed this activity's speaker and planner disclosures and resolved all identified conflicts of interest, if applicable.

Special Assistance

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

mark your calendars!

ASA 35th Annual Conference
April 10 – 13, 2010
Omni Houston Hotel
Houston, Texas

Andrology Lab Workshop
April 10, 2010

Special Symposium
April 10, 2010

schedule of events

XX North American Testis Workshop
Testicular Function: Levels of Regulation
April 1 – 4, 2009

Chair: Mitch Eddy, PhD
Vice-Chair: John McCarrey, PhD
Location: Columbus Ballroom, 2nd Floor

WEDNESDAY, APRIL 1, 2009

6:00 p.m. – 8:30 p.m. **Registration**

7:00 p.m. – 7:15 p.m. **Welcome**
Mitch Eddy PhD

7:15 p.m. – 8:30 p.m. **Keynote Address: Testicular Function and Dysfunction in Mammals**
Martin Matzuk, MD, PhD
Baylor College of Medicine

THURSDAY, APRIL 2, 2009

7:30 a.m. – 6:00 p.m. **Registration**

7:30 a.m. – 8:30 a.m. **Continental Breakfast**

SESSION I: REGULATION OF TESTIS DEVELOPMENT
Chair: Ina Dobrinski, University of Pennsylvania

8:30 a.m. – 9:30 a.m. **Benchmark Lecture: Cellular Mechanisms of Testis Formation**
Richard Behringer, PhD
University of Texas MD Anderson Cancer Center

9:30 a.m. – 10:15 a.m. **Multifaceted Functions of Hedgehog Pathway in Fetal Testis Development**
Humphrey Yao, PhD
University of Illinois

10:15 a.m. – 10:45 a.m. **Break**

10:45 a.m. – 11:30 a.m. **Determination and Stability of Sex**
David Schlessinger, PhD
National Institute of Aging, NIH

11:30 a.m. – 12:15 p.m. **Sertoli Cell Dicer is Essential for Spermatogenesis**
Serge Nef, PhD
University of Geneva, Switzerland

12:15 p.m. – 1:30 p.m. **Break**

SESSION II: REGULATION OF TESTIS FUNCTION
Chair: Tony Plant, University of Pittsburgh

1:30 p.m. – 2:15 p.m. **Germ Cells: What They Need and Why**
Sarah J. Meachem, PhD
Prince Henry's Institute of Medical Research
Melbourne, Australia

2:15 p.m. – 3:00 p.m. **Gonadotrophin-Regulated Testicular RNA Helicase, GRTH/Ddx25, an Essential Multifunctional Protein in Germ Cell Development**
Chon-Hwa Tsai-Morris, PhD
NICHD, NIH

3:00 p.m. – 3:30 p.m. **Break**

3:30 p.m. – 4:15 p.m. **The Role of A-Kinase Anchoring Protein 121 (AKAP121) in the Regulation of Steroid Hormone Biosynthesis**
Douglas M. Stocco, PhD
Texas Tech University

SHORT TALKS FROM SUBMITTED ABSTRACTS – I
Chair: Mary Ann Handel, The Jackson Laboratory

4:15 p.m. – 4:30 p.m. **Short Talk #1**

4:30 p.m. – 4:45 p.m. **Short Talk #2**

4:45 p.m. – 5:00 p.m. **Short Talk #3**

5:00 p.m. **Poster Session**

FRIDAY, APRIL 3, 2009

7:30 a.m. – 6:00 p.m. **Registration**

7:30 a.m. – 8:30 a.m. **Continental Breakfast**

SESSION III: REGULATION OF GERM CELL DEVELOPMENT
Chair: John McCarrey, University of Texas San Antonio

8:30 a.m. – 9:30 a.m. **Benchmark Lecture: P-Granule Assembly in C. Elegans Germ Cells**
Dustin Updike, PhD
University of California, Santa Cruz

9:30 a.m. – 10:15 a.m. **Germ Line Stem Cell Renewal and Differentiation in the Mouse**
Makoto Nagano, PhD
McGill University

10:15 a.m. – 10:45 a.m. **Break**

10:45 a.m. – 11:30 a.m. **The Role of the Chromatoid Body in RNA Processing in Male Germ Cells**
Paolo Sassone-Corsi, PhD
University of California – Irvine

11:30 a.m. – 12:15 p.m. **Epigenetic Control of Primordial Germ-Cell Specific Gene Expression**
Yasuhisa Matsui, PhD
Tohoku University, Sendai, Japan

12:15 p.m. – 1:30 p.m. **Break**

SESSION IV: REGULATION OF GENE EXPESSION IN GERM CELLS
Chair: Norman Hecht, University of Pennsylvania

1:30 p.m. – 2:15 p.m. **Sam68 Nuclear RNA-Binding Protein and its Role During Spermatogenesis**
Claudio Sette, PhD
University of Rome "Tor Vergata"

2:15 p.m. – 3:00 p.m. **Small RNA Pathways in Male Germline**
Alexei Aravin, PhD
Cold Spring Harbor Laboratory

3:00 p.m. – 3:30 p.m. **Break**

3:30 p.m. – 4:15 p.m. **Genome Divergence and Male Infertility in Mouse**
Fernando Pardo-Manuel de Villena, PhD
University of North Carolina

SHORT TALKS FROM SUBMITTED ABSTRACTS – II
Chair: Robert Braun, The Jackson Laboratory

4:15 p.m. – 4:30 p.m.	**Short Talk #4**
4:30 p.m. – 4:45 p.m.	**Short Talk #5**
4:45 p.m. – 5:00 p.m.	**Short Talk #6**
5:00 p.m.	**Poster Session II**

SATURDAY, APRIL 4, 2009

7:30 a.m. – 8:30 a.m.	**Coffee**
7:00 a.m. – 7:00 p.m.	**Registration**

SESSION V: REGULATION OF GAMETE DEVELOPMENT AND FUNCTION
Chair: Debbie O'Brien, University of North Carolina

8:30 a.m. – 9:15 a.m.	**Histone Demethylase JHDM2A in Post-Meiotic Gene Expression in the Mouse** Yuki Okada, PhD University of North Carolina
9:15 a.m. – 10:00 a.m.	**Regulation of Male Meiosis by the X Chromosome-Linked Genes in Mice** P. Jeremy Wang, MD, PhD University of Pennsylvania
10:00 a.m. – 10:30 a.m.	**Break**
10:30 a.m. – 11:15 a.m.	**Building Germ Cell-Specific Signaling Complexes that Regulate Sperm Function** Marco Conti, MD University of California San Francisco
11:15 a.m. – 12:00 p.m.	**LDHC: The Ultimate Model of Testis Gene Expression** Erv Goldberg, PhD Northwestern University
12:00 p.m.	**Recess**

Andrology Lab Workshop
Sperm Morphology: A Hands-On Workshop With a Novel Morphology Consensus Workshop

Chair: Angela Reese, TS
Location: Riverview Room AB, 3rd Floor

SATURDAY, APRIL 4, 2009

Sperm Morphology Workshop I: Morphology Classification and Quality Control

8:00 a.m. – 8:30 a.m.	**Continental Breakfast**
8:30 a.m. – 9:15 a.m.	**Overview of Sperm Morphology Classification Systems** Susan Rothmann, PhD, HCLD Fertility Solutions Inc.
9:15 a.m. – 10:00 a.m.	**Interactive Instruction – WHO 3rd Edition and Strict/WHO 4th Edition Classification Methods** Aniela Bollendorf, MT Cooper Institute for Reproductive Hormonal Disorders Angela Reese, TS Fertility Solutions Inc.
10:00 a.m. – 11:30 a.m	**Exercise 1 – Sperm Morphology** *Refreshment break included; participants will take break as needed.* Aniela Bollendorf, MT Cooper Institute for Reproductive Hormonal Disorders Angela Reese, TS Fertility Solutions Inc.
11:45 a.m. – 1:15 p.m.	**Laboratory Science Forum Luncheon (included)** **Leukocytes and Spermatocytes: Seminal Clues to Reproductive Health** Charles H. Muller, PhD, HCLD University of Washington School of Medicine
1:30 p.m. – 2:15 p.m.	**Exercise 2 – Sperm Morphology** Aniela Bollendorf, MT Cooper Institute for Reproductive Hormonal Disorders Angela Reese, TS Fertility Solutions Inc.
2:15 p.m. – 3:00 p.m.	**Quality Control – Understanding QC Requirements and Construction & Use of Control Charts** Steve Schrader, PhD NIOSH Susan Rothmann, PhD, HCLD Fertility Solutions Inc.
3:00 p.m. – 3:15 p.m.	**Refreshment Break**
3:15 p.m. – 3:45 p.m.	**Clinical Case Studies** David Karabinus, PhD, HCLD Genetics and IVF Institute
3:45 p.m. – 4:15 p.m.	**Exercise 3 – How-to Quality Control Exercise**
4:15 p.m. – 4:30 p.m.	**Q&A**

SUNDAY, APRIL 5, 2009

Sperm Morphology Workshop II: Morphology Consensus Building

8:00 a.m. – 8:10 a.m.	**Introduction** Susan Rothmann, PhD, HCLD Fertility Solutions Inc. Steve Simon, PhD P. Mean Consulting Dean Morbeck, PhD Mayo Clinic
8:10 a.m. – 9:00 a.m.	**Morphology Consensus Study Part I**
9:00 a.m. – 9:30 a.m.	**Distinguished Service Award / Refreshment Break** *Location: Columbus Ballroom, 2nd Floor*
9:30 a.m. – 10:00 a.m.	**Discussion of Data & Controversial Images** Steve Simon, PhD P. Mean Consulting
10:00 a.m. – 10:30 a.m.	**Morphology Consensus Study Part II**
10:30 a.m. – 10:45 a.m.	**Break**
10:45 a.m. – 11:45 a.m.	**Discussion of Consensus Data** Steve Simon, PhD P. Mean Consulting
11:45 a.m. – 12:00 p.m.	**Open Discussion of Future Work, Recommendations**

*Locations subjects to change

schedule of events

ASA Special Symposium
Issues In Urologic and Hormonal Health

Chair: Arthur L. Burnett, MD
Location: Columbus Ballroom, 2nd Floor

Registration is complimentary; however, limited seating is available.

1:00 p.m. – 1:30 p.m.	**Testosterone and Bone Health** Lisa Tenover, MD, PhD Emory University, Wesley Woods Health Center
1:30 p.m. – 2:00 p.m.	**Testosterone and Other Therapies for Penile Health After Radical Prostatectomy** Andrew McCullough, MD NYU Urology Association
2:00 p.m. – 2:20 p.m.	**Q&A**
2:20 p.m. – 2:50 p.m.	**5-Alpha Reductase Inhibitor Effects on Prostate and Sexual Function** Stephanie Page, MD, PhD University of Washington
2:50 p.m. – 3:20 p.m.	**Androgen Replacement and Prostate Cancer** Abraham Morgentaler, MD Men's Health Boston
3:20 p.m. – 3:40 p.m.	**Q&A**
3:40 p.m. – 3:50 p.m.	**Break**
3:50 p.m. – 4:20 p.m.	**BPH/LUTS and Erectile Dysfunction: Nexus and Phosphodiesterase-5 Inhibitor Role** Ajay Nehra, MD Mayo Clinic
4:20 p.m. – 4:50 p.m.	**Intralesional and Surgical Therapy for Peyronie's Disease** Wayne Hellstrom, MD Tulane University School of Medicine
4:50 p.m. – 5:10 p.m.	**Q&A**

ASA 34th Annual Meeting
"Bench to Bedside: Advances in Andrology"
April 4 – 7, 2009

Program Chairs: Dolores Lamb, PhD
Dana Ohl, MD

*All sessions will be located in the **Columbus Ballroom** unless otherwise noted.*
Registration fee includes entry into the lectures, one ticket to the Welcome Reception, a syllabus, and refreshment break.

FRIDAY, APRIL 3, 2009

7:00 a.m. – 6:00 p.m.	**Registration** *Location: Columbus Ballroom Foyer, 2nd Floor*

SATURDAY, APRIL 4, 2009

7:00 a.m. – 7:00 p.m.	**Registration** *Location: Columbus Ballroom Foyer, 2nd Floor*
4:00 p.m. – 9:30 p.m.	**Exhibit Hall Open** *Location: Grand Ballroom, 2nd Floor*
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.	**ASA KEYNOTE LECTURE** **Sex Determination and Fetal Testis Development** *Blanche Capel, PhD* *Duke University School of Medicine* *(Introduced by Wayne J.G. Hellstrom, MD)*
7:30 p.m. – 7:45 p.m.	**Updates from NICHD** *Stuart Moss, PhD* *NICHD*
7:45 p.m. – 9:30 p.m.	**Welcome Reception** *Location: Grand Ballroom, 2nd Floor*

SUNDAY, APRIL 5, 2009

6:30 a.m. – 8:00 a.m.	**Past President's Breakfast** *Location: Riverview A, 3rd Floor*
7:00 a.m. – 4:00 p.m.	**Exhibit Hall Open** *Location: Grand Ballroom, 2nd Floor*
7:00 a.m. – 6:00 p.m.	**Registration** *Location: Columbus Ballroom Foyer, 2nd Floor*
8:00 a.m. – 9:00 a.m.	**WOMEN IN ANDROLOGY LECTURE** **Androgen Receptor Variation and Prostate Cancer in Humanized AR Mice** *Diane Robins, PhD* *University of Michigan* *(Introduced by Kate Loveland, PhD)*
9:00 a.m. – 9:15 a.m.	**Distinguished Service Award**
9:15 a.m. – 9:30 a.m.	**Quick Break**
9:30 a.m. – 11:00 a.m.	**SYMPOSIUM I – Meiosis in the Male** *Co-Chairs: Patricia L. Morris, PhD* *Petrice Brown, PhD* **Mismatch Repair Proteins in Male Meiosis** *Paula Cohen, PhD* *Cornell University* **Recombination, DNA Damage Repair and Meiosis** *Anton Grootegoed, PhD* *Rotterdam, The Netherlands* **MutS Homologues (hMSH4-hMSH5) Interactions During Meiosis** *Richard Fishel, PhD* *Ohio State University Medical Center*
11:00 a.m. – 12:30 p.m.	**Poster Session I** *Location: Grand Ballroom, 2nd Floor*

*Locations subjects to change

schedule of events

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**
"Shaping Our Research Communities: What Women Do Well and How to Do it Better!"
(Not included in registration fee; tickets required.)
Location: Riverview B, 3rd Floor
Joan M. Lakoski, PhD

2:00 p.m. – 3:30 p.m. **Concurrent Oral Session I**

Basic Research
Moderators: Barry Hinton, PhD
Sophie-Ann Lamour, BSc

Location: Columbus Ballroom, 2nd Floor

2:00 p.m. – Abstract 1
IGFBP-3 ENHANCES GERM CELL APOPTOSIS INDUCED BY TESTICULAR HORMONAL DEPRIVATION
YanHe Lue, MD, Hemal Mehta, PhD, QingHai Liu, MD, Pinchas Cohen, MD, Yue Jia, PhD, Amiya Sinha Hikim, PhD, Ronald Swerdloff, MD and Christina Wang, MD (Presented By: YanHe Lue)

2:15 p.m. – Abstract 2
EFFECTS OF DI- (2-ETHYLHEXYL) PHTHALATE ON RAT LEYDIG CELL REGENERATION
Xingwang Li, PhD, MD, Qing-Quan Lian, PhD, MD and Ren-Shan Ge, MD (Presented By: Xingwang Li)

2:30 p.m. – Abstract 3
ABSENCE OF ESTROGEN RECEPTOR ALPHA LEADS TO A MISREGULATION OF THE EPIDIDYMAL FLUID MILIEU AND A CONSEQUENT DEFECT IN SPERM FUNCTION
Avenel Joseph, Rex Hess, PhD, Chemyong Ko, PhD and Barry Shur, PhD (Presented By: Avenel Joseph)

2:45 p.m. – Abstract 4
A NOVEL MOUSE MODEL OF ASTHENOSPERMIA
Claire Borg, Victoria Adams, David de Kretser and Moira O'Bryan (Presented By: Claire Borg)

3:00 p.m. – Abstract 5
JAM-A INTERACTS WITH CASK AND PMCA4 IN THE PLASMA MEMBRANE AND REGULATES CA2+ HOMEOSTASIS AND MURINE SPERM MOTILITY
Rolands Aravindan, PhD, Ulhas Naik, PhD, Victor Fomin, PhD, Randall Duncan, PhD, Deni Galileo, PhD and Patricia Martin-DeLeon, PhD (Presented By: Rolands Aravindan)

3:15 p.m. – Abstract 6
DNA BINDING ACTIVITY OF HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) IN THE NORMOXIC AND ISCHEMIC TESTIS
Anoop Shah, Rebecca Tyson and Michael Palladino, BS, PhD (Presented By: Anoop Shah)

2:00 p.m. – 3:30 p.m. **Concurrent Oral Session II**

Clinical Research
Moderators: Robert Oates, MD
Ajay Nangia, MBBS

Location: Washington Room, 3rd Floor

2:00 p.m. – Abstract 7
GENOME-WIDE SCAN FOR SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ASSOCIATED WITH SEVERE OLIGOZOOSPERMIA AND AZOOSPERMIA IN MEN
Kenneth Aston, PhD and Douglas Carrell, PhD (Presented By: Kenneth Aston)

2:15 p.m. – Abstract 8
RACIAL DIFFERENCES IN VASECTOMY UTILIZATION IN THE UNITED STATES: DATA FROM THE NATIONAL SURVEY OF FAMILY GROWTH
Michael Eisenberg, MD, Jillian Henderson, PhD, MPH, JohnAmory, MD, MPH, James Smith, MD, MS and Thomas Walsh, MD, MS (Presented By: Michael Eisenberg)

2:30 p.m. – Abstract 9
THE LONGITUDINAL EFFECTS ON PENILE OXYGEN SATURATION: A PROSPECTIVE RANDOMIZED STUDY OF THE NIGHTLY USE OF INTRAURETHRAL ALPROSTADIL VS SILDENAFIL FOLLOWING NERVE SPARING RADICAL PROSTATECTOMY (NSRP)
Joseph Alukal, MD, Brianne Goodwin, BS, Artrit Bytyci, BS and Andrew McCullough, MD (Presented By: Joseph Alukal)

2:45 p.m. – Abstract 10
REAL-TIME PERIPROSTATIC TISSUE IMAGING WITH MULTIPHOTON MICROSCOPY FOR IMPROVING POTENCY OUTCOMES DURING NERVE-SPARING RADICAL PROSTATECTOMY: INITIAL RESULTS FROM FRESH HUMAN PROSTATECTOMY SPECIMENS
Gerald Tan, MB, ChB, MRCSEd, MMed, FAMS, Rajiv Yadav, MD, Michael Herman, MD, Sushmita Mukherjee, PhD, Frederick Maxfield, PhD, Watt Webb, DSc and Ashutosh Tewari, MD, MCh (Presented By: Gerald Tan)

3:00 p.m. – Abstract 11
EFFECTS OF LIFESTYLE EXPOSURES AND BODY MASS INDEX ON SPERM QUALITY PARAMETERS OF FERTILE MEN
Karlene Lavelle, PhD, RN, Andrew Olshan, PhD, Amy Herring, ScD, David Savitz, PhD, Anna Maria Siega-Riz, PhD and Sally Perreault, PhD (Presented By: Karlene Lavelle)

3:15 p.m. – Abstract 12
DEVELOPMENT OF A NOVEL MICROSURGICAL MOUSE MODEL FOR THE STUDY OF VARICOCELE PATHOPHYSIOLOGY
Howard Kim, MD, Philip Li, MD, Chantal Sottas, Renshan Ge, MD and Marc Goldstein, MD (Presented By: Howard Kim)

3:30 p.m. – 3:45 p.m. **Refreshment Break**
Location: Grand Ballroom, 2nd Floor

3:45 p.m. – 4:30 p.m. **LECTURE I**
Prostate Stem Cells: Friend or Foe
Susan Kasper, PhD
Vanderbilt University Medical Center
(Introduced by Gail S. Prins, PhD)

schedule of events

4:30 p.m. – 5:15 p.m.
EAA LECTURE
The Y Chromosome Side of Male Fertility
Csilla Krausz, MD, PhD
University of Florence
(Introduced by Patricia Cuasnicu, PhD)

5:15 p.m. – 5:45 p.m.
CLINICAL LECTURE
Why Genetic Testing is Required in the Evaluation of the Severely Oligospermic / Azoospermic Male
Robert Oates, MD
Boston University
(Introduced by Dolores Lamb, 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: Washington Room, 3rd Floor

MONDAY, APRIL 6, 2009

7:00 a.m. – 12:30 p.m.
Exhibit Hall Open
Location: Grand Ballroom, 2nd Floor

7:00 a.m. – 6:00 p.m.
Registration
Location: Columbus Ballroom Foyer, 3rd Floor

8:00 a.m. – 9:00 a.m.
AUA LECTURE
Hypogonadism – Assessment of Quality of Life and New Tools for Clinical and Research Refinement
Raymond Rosen, PhD
New England Research Institutes
(Introduced by Dana A. Ohl, MD)

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

9:15 a.m. – 9:30 a.m.
Break

9:30 a.m. – 11:00 a.m.
SYMPOSIUM II – Regulation of Male Fertility: The Yin and the Yang
Co-Chairs: Janice P. Evans, PhD
Matthew Marcello, BS

Can a Safe, Effective, Reversible Male Contraceptive be Developed?
John Amory, MD
University of Washington

Properties of Spermatogonial Stem Cells
Dirk de Rooij, PhD
Utrecht University, The Netherlands

PLCzeta and Male Infertility
Rafael Fissore, PhD
University of Massachusetts

11:00 a.m. – 12:30 p.m.
Poster Session II
Location: Grand Ballroom, 2nd Floor

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

12:30 – Committee

12:30 p.m. – 2:00 p.m.
MENTORING LUNCHEON SPONSORED BY THE DIVERSITYAND TRAINEE AFFAIRS COMMITTEES
(Not included in registration fee; tickets required.)
Location: Riverview B, 3rd Floor
"Using PowerPoint Without PowerPoint Using You"
Barry T. Hinton, PhD
University of Virginia School of Medicine

12:30 p.m. – 2:00 p.m.
Editorial Board Luncheon
Location: Riverview A, 3rd Floor

2:00 p.m. – 3:00 p.m.
THE ASA INTERNATIONAL LECTURE
Homeobox Genes in Mammalian Sexual Development
Peter Koopman, PhD
University of Queensland
(Introduced by Patricia S. Cuasnicu, PhD)

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

3:30 p.m. – 5:00 p.m.
SYMPOSIUM III – Ejaculation and Ejaculatory Dysfunction
Co-Chairs: Peter Chan, MD
Darius A. Paduch, MD, PhD

Central and Peripheral Control of Ejaculation
Francois Giuliano, MD
University of Paris West

Premature Ejaculation- Epidemiology and Treatment
Wayne Hellstrom, MD
Tulane University Medical School

Current Trends in the Treatment of Infertility in Men with Spinal Cord Injury
Nancy Brackett, PhD
The Miami Project to Cure Paralysis

5:00 p.m.
ASA Business Meeting, Outstanding Trainee Investigator and Trainee Awards

7:00 p.m. – 11:00 p.m.
Annual Banquet – Independence Seaport Museum
(Not included in registration fee; tickets required.)
Location: Independence Seaport Museum

TUESDAY, APRIL 7, 2009

7:00 a.m. – 8:00 a.m.
2010 Program Committee Meeting
Location: USS New Jersey, 2nd Floor

7:30 a.m. – 12:15 p.m.
Registration
Location: Columbus Ballroom, 2nd Floor

schedule of events

8:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Advances in Andrology
Co-Chairs: David Karabinus, PhD
Joseph Alukal, MD

Single-Cell Analysis of Androgen Responses
Michael Mancini, PhD
Baylor College of Medicine

Essential Function of the First Bromodomain Motif in Bromodomain Protein for Spermiogenesis in Mice and Men
Debra J. Wolgemuth, PhD
College of Physicians and Surgeons of Columbia University

Microfluidic Applications in Andrology
Gary Smith, PhD
University of Michigan

9:30 a.m. – 10:00 a.m.

Refreshment Break
Location: Columbus Ballroom Foyer, 2nd Floor

10:00 a.m. – 10:45 a.m.

LECTURE II:
Male Circumcision and HIV/ST1 Infection Risk in Men and Women
Ronald H. Gray, PhD
Johns Hopkins University
(Introduced by Kirk C. Lo, MD, FRCSC)

10:45 a.m. – 12:15 p.m.

SYMPOSIUM V – Excurrent Genital Tract
Co-Chairs: Terry T. Turner, PhD
Joel Marmar, MD

Tubular Morphogenesis of the Developing Wolffian / Epididymal Duct: More Twists And Turns
Barry Hinton, PhD
University of Virginia School of Medicine

Amyloid in the Reproductive Tract: Pathology or Biological Function?
Gail Cornwall, PhD
Texas Tech School of Medicine

Post-Partum Initiation of Definition of Transcription in Various Epididymal Regions
John Herr, PhD
University of Virginia School of Medicine

MEETING ADJOURNED

poster session 1

Sunday, April 5, 2009
11:00 a.m. – 12:30 p.m.
Location: Grand Ballroom, 2nd Floor

SPERM FUNCTION / SEMEN ANALYSIS

POSTER 13 **DETECTION OF SOLUBLE ADENYLYL CYCLASE (ADCY10) HOMOLOG PROTEINS IN BOAR SPERMATOZOA**
Kazumi Nakamura, BS, Chihiro Suzuki, BS, Shunsuke Tate, MS, Jibak Lee, PhD and Hiroshi Harayama, PhD (Presented By: Hiroshi Harayama)

POSTER 14 **SMALL UBIQUITIN-RELATED MODIFIER-1 (SUMO-1) IN EJACULATED SPERMATOZOA: A NEW MODULATING FACTOR OF SPERM MOTILITY?**
Monica Muratori, Lucia Giuliano, PhD student, Sara Marchiani, PhD, Lara Tamburrino, PhD student, Daniele Nosi, PhD, Gianni Forti, MD and Elisabetta Baldi, PhD (Presented By: Monica Muratori)

POSTER 15 **ARE ALL CREATED EQUAL? IS IT POSSIBLE TO "SUPER-SELECT" THE MOST VIABLE ONES FROM A SAMPLE OF SPERM WITH MINIMAL MOTILITY?**
Maurice Garcia, MD, Aaron Ohta, MD, Thomas Walsh, MD, James Smith, MD, Ming Wu, MD and Tom Lue, MD (Presented By: Maurice Garcia)

POSTER 16 **EFFECT OF MUCUNA PRUREINS ON INTRACELLULAR REACTIVE OXYGEN SPECIES, STRUCTURAL AND FUNCTIONAL INTEGRITY OF EPIDIDYMAL SPERMATOZOA IN AGED RAT**
Seppan Prakash, MSc, PhD, Sekar Suresh, MSc and Elumalai Prithiviraj, MSc (Presented By: Seppan Prakash)

POSTER 17 **NA+K+-ATPASE AND CAPACITATION SIGNALLING IN BOAR SPERMATOZOA**
Meghan Mackenzie Bell, BSc and Mary Buhr, PhD (Presented By: Meghan Mackenzie Bell)

POSTER 18 **REACTIVE OXYGEN SPECIES AND BOAR SPERM FUNCTION**
Basim Awda, PhD, Meghan Bell, BSc and Mary Buhr, PhD (Presented By: Basim Awda)

POSTER 19 **GENOME-WIDE APPROACHES TO IDENTIFYING SPERM BIOMARKERS OF TOXICANT EXPOSURE**
Sara Pacheco, BS and Kim Boekelheide, MD, PHD (Presented By: Sara Pacheco)

POSTER 20 **UBIQUITOUS HYALURONIDASES ARE PRESENT IN HUMAN AND MOUSE SPERM WHERE THEY ARE ENZYMATICALLY ACTIVE**
Kristen Reese, BS, Rolands Aravindan, PhD, Griffiths Genevieve, PhD, Shao Minghai, PhD, Bruton Jessica, BS, Woody Sarah, BS, Barran Diniece, BS, Naik Tejal, BS, Galileo Deni, PhD and Patricia Martin-DeLeon, PhD (Presented By: Patricia Martin-DeLeon)

POSTER 21 **ADJUDIN IS A PROSPECTIVE CANDIDATE OF SPERMICIDE**
Hui Wang, PhD Candidate, Xiangxiang Chen, Master, Zuomin Zhou, PhD, Xuejiang Guo, PhD, Fuqiang Wang, Master, Jing Xie, Master and Jiahao Sha, PhD (Presented By: Hui Wang)

POSTER 22 **VAMP4, A SPERM MOTILITY RELATED V-SNARE PROTEIN**
Jun Xing, PhD candidate, Jian Shen, PhD candidate, Xuejiang Guo, PhD candidate, Zuoming Zhou, PhD[2] and Jiahao Sha, PhD[2] (Presented By: Jun Xing)

POSTER 23 **CAN SPERM CHROMATIN PACKAGING INFLUENCE RESULTS OF SPERM DNA INTEGRITY MEASURES? RESULTS OF THE TUNEL ASSAY**
Madelaine From Björk, Biomedical Scientist, Lars Björndahl, MD PhD, Alex Zakeri, Biomedical Scientist and Ulrik Kvist, MD PhD (Presented By: Lars Björndahl)

POSTER 24 **THE IMPACT OF CIGARETTE SMOKING ON SPERM DNAAND OXIDATIVE STRESS IN NON-LEUKOCYTOSPERMIC INFERTILE MEN**
Moustafa Elsaied, MD, Mohamed Elshal, PhD, Ibrahim El-Sayed, PhD and Samir Elmasry, PhD (Presented By: Moustafa Elsaied)

POSTER 25 **PROSPECTIVE STUDY OF FERTILAID VITAMIN IN MEN WITH LOW SPERM QUALITY**
G. Dennis Clifton, BS, PharmD and J.E. Ellington, DVM, PhD (Presented By: G. Dennis Clifton)

POSTER 26 **DEATH OF HUMAN SPERMATOZOA MAY BE REGULATED SEPARATELY BY MITOCHONDRIAL AND CYTOSOLIC PATHWAYS**
Lisa Welch, MSc and Samuel Prien, PhD (Presented By: Lisa Welch)

POSTER 27 **CAN SPERM CHROMATIN PACKAGING INFLUENCE RESULTS OF SPERM DNA INTEGRITY MEASURES? RESULTS OF THE COMET ASSAY**
Jessica Tu, Biomedical Scientist, Lars Björndahl, MD PhD and Ulrik Kvist, MD PhD (Presented By: Lars Björndahl)

POSTER 28 **OBJECTIVE COMPARISON OF THE SPERM CLASS ANALYZER® TO MANUAL ASSESSMENT FOR GRADING HUMAN SPERM MORPHOLOGY BY STRICT CRITERIA**
Michael Klug, BS, Jolene Fredrickson, MS, Anthony Krenik, BS and Dean Morbeck, PhD (Presented By: Michael Klug)

POSTER 29 **TYROSINE PHOSPHORYLATION OF ACIDIC PROTEINS LOCALIZED TO THE ACROSOME IS ASSOCIATED WITH COLD SHOCK- AND CALCIUM IONOPHORE-INDUCED ACROSOME REACTIONS IN PORCINE SPERM**
Hannah Galantino-Homer, VMD, PhD, DACT, Mark Modelski, BS and Ina Dobrinski, Drmedvet, MVSc, PhD, DACT
(Presented By: Hannah Galantino-Homer)

POSTER 30 **EJACULATE TRAITS AND SEMINAL PLASMA CHEMISTRY OF GOOD VERSUS POOR QUALITY EJACULATES IN ASIAN ELEPHANTS (ELEPHAS MAXIMUS)**
Wendy Kiso, MNAS, Dennis Schmitt, DVM, PhD, DACT, Janine Brown, PhD and Budhan Pukazhenthi, BVSc, PhD
(Presented By: Wendy Kiso)

POSTER31 **COMPARISON OF SPERM DNA INTEGRITY BEFORE AND AFTER DENSITY GRADIENT CENTRIFUGATION EMPLOYING A NOVEL TOLUIDINE BLUE ASSAY**
Dennis Marchesi, Hannah Biederman, BS, Scott Ferrara, N/A, Huailiang Feng, PhD and Avner Hershlag, MD
(Presented By: Dennis Marchesi)

POSTER32 **CHARACTERIZATION OF THE BIOCHEMICAL PROCESSES OF CAPACITATION AND CRYOCAPACITATION AND THE ROLE OF SEMINAL PLASMA IN PORCINE SPERM CELLS**
Melissa Vadnais and Gary Althouse, DVM, MS, PhD (Presented By: Melissa Vadnais)

POSTER 33 **ROS LEVELS ARE INDEPENDENT OS SPERM CONCENTRATION, MOTILITY AND ABSTINENCE IN NORMAL, HEALTHY PROVEN FERTILE MAN – A LONGUTIDINAL STUDY**
Nisarg Desai, MD, Rakesh Sharma, PhD, Reda Mahfouz, MD, Sajal Gupta, MD, Edmund Sabanegh, MD and Ashok Agarwal, PhD
(Presented By: Nisarg Desai)

POSTER 34 **HUMAN SPERM DNA INTEGRITY: CORRELATION BETWEEN THE TUNEL ASSAY AND A NOVEL TOLUIDINE BLUE ASSAY**
Dennis Marchesi, Hannah Biederman, BS, Scott Ferrara, N/A, Huiliang Feng, PhD and Avner Hershlag, MD (Presented By: Dennis Marchesi)

POSTER35 **RELEVANCE OF MORPHOLOGIC HETEROGENEITY IN HUMAN AND ANIMAL SPERM**
Sonja Grunewald, MD, Malte Strittmatter, MS, Hans-Wilhelm Michelmann, PhD, Peter Schwartz, PhD, Hans-Juergen Glander, PhD and Uwe Paasch, PhD (Presented By: Sonja Grunewald)

POSTER36 **COMPARISON OF SPERM CHROMATIN DISPERSION TEST AND TUNEL ASSAYS TO ASSESS THE DNA FRAGMENTATION IN HUMAN SPERM**
Lihong Zhang, Master, Yi Qiu, Bachelor, Kehua Wang, PhD, Leiguang Wang, Bachelor, Juan Li and Meixin Zhang
(Presented By: Lihong Zhang)

POSTER 37 **EFFECTS OF VARDENAFIL ON SPERM CAPACITY TO UNDERGO HYPERACTIVATION**
Evlalia Vlachopoulou, BS, Dimitrios Baltogiannis, MD, PhD, Michalis Rimikis, MD, Stavros Tsambalas, MD, PhD, Stavros Gratsias, MD and Nikolaos Sofikitis, Professor (Presented By: Nikolaos Sofikitis)

FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT

POSTER 38 **EVIDENCE FOR THE PARTICIPATION OF HUMAN EPIDIDYMAL PROTEIN CRISP1 IN SPERM-ZONA PELLUCIDA INTERACTION**
Julieta A. Maldera, Dolores Busso, PhD, Mayel Chirinos, PhD, Fernanda G. Raffo, Jorge A. Blaquier, PhD, Fernando Larrea, PhD and Patricia S. Cuasnicu, PhD (Presented By: Julieta A. Maldera)

POSTER39 **PARTICIPATION OF EPIDIDYMOSOMES IN THE ASSOCIATION OF EPIDIDYMAL PROTEIN CRISP1 WITH SPERMATOZOA**
Julieta A. Maldera, Miguel W. Fornés, PhD, Débora J. Cohen, PhD, Juan I. Enesto, Gustavo Vasen and Patricia S. Cuasnicu, PhD
(Presented By: Julieta A. Maldera)

POSTER40 **FUNCTIONAL CONSEQUENCES OF CLEAVAGE, DISSOCIATION, AND EXOCYTOTIC RELEASE OF ZP3R/SP56 FROM THE MOUSE SPERM ACROSOMAL MATRIX**
Mariano Buffone, PhD, Kye-Seong Kim, PhD, Birgit Doak, MS, Esmeralda Rodriguez-Miranda, PhD and George Gerton, PhD (Presented By: Mariano Buffone)

INFERTILITY / ART / MALE CONTRACEPTION

POSTER41 **A NOVEL MOUSE MODEL OF ASTHENOSPERMIA**
Claire Borg, Victoria Adams, David de Kretser and Moira O'Bryan (Presented By: Claire Borg)

POSTER42 **INITIAL SEMEN ANALYSIS WITH AZOOSPERMIA IS PREDICTIVE OF SUCCESSFUL VASECTOMY FOR STERILIZATION**
Run Wang, MD, Haocheng Lin, MD and Yutian Dai, MD (Presented By: Run Wang)

POSTER43 **INFERTILITY AND ABNORMAL SPERM IN MICE LACKING THE HIP/RPL29 GENE**
Rolands Aravindan, PhD, Michelle Smith, BA, Catherine Kim-Safran, PhD, Daniel Carson, PhD and Patricia Martin-DeLeon, PhD
(Presented By: Rolands Aravindan)

POSTER44 **FIRST REPORT ON THE ABSENCE OF VIRAL LOAD AND USE OF TESTICULAR SPERM SAMPLES OBTAINED FROM MALES WITH HEPATITIS C AND HIV AFTER WASH**
Nicolas Garrido, PhD, Manuel Gil-Salom, MD, Jose M. Martinez-Jabaloyas, MD and Marcos Meseguer, PhD
(Presented By: Nicolas Garrido)

poster session 1

POSTER 45 **MOLECULAR PREDICTORS FOR INFERTILITY IN PATIENTS WITH VARICOCELES: A ROLE FOR CYCLIC AMP-RESPONSE ELEMENT MODULATOR (CREM)**
Susan Benoff, PhD, Ian Hurley, PhD, Barbara Napolitano, MA and Joel Marmar, MD (Presented By: Susan Benoff)

POSTER 46 **A NOVEL NON-INVASIVE, MOTILITY-INDEPENDENT SPERM SORTING METHOD AND TECHNOLOGY TO ISOLATE AND RETRIEVE VIABLE SPERM FROM NON-VIABLE SPERM, FOR USE WITH ISCI**
Maurice Garcia, MD, Aaron Ohta, PhD, Thomas Walsh, MD, Ming Wu, PhD and Tom Lue, MD (Presented By: Maurice Garcia)

POSTER 47 **THE RARE ADDRA GAZELLE (GAZELLA DAMA RUFICOLLIS) PRODUCES VIABLE SPERMIC EJACULATES THAT TOLERATE CRYOPRESERVATION**
Budhan Pukazhenthi, BVSc, PhD, Pierre Comizzoli, DVM, PhD, Luis Padilla, DVM, Nucharin Songsasen, DVM, PhD, Samantha Favorretto, BS and David Wildt, PhD (Presented By: Budhan Pukazhenthi)

POSTER 48 **VASECTOMY BY EPITHELIAL CURETTAGE WITHOUT SUTURE OR CAUTERY: A PILOT STUDY IN MAN**
John Amory, MD, MPH, John Jessen, DDS, William Bremner, MD, PhD and Richard Berger, MD (Presented By: John Amory)

POSTER 49 **THE HYALURONAN BINDING ASSAY (HBA): A STATISTICALLY SIGNIFICANT AND ROBUST DIAGNOSTIC ASSAY IN DIRECTING ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) TREATMENT AND PREDICTING CLINICAL PREGNANCY (CP)**
Kathryn Worrilow, PhD, Chizoba Uzochukwu, MSc, DO, Jaime Bower, BS, Marsha Wender-Timmerman, MSc and Sherrine Eid, MPH (Presented By: Kathryn Worrilow)

POSTER 50 **EMBOLIZATION VERSUS MICROSURGICAL VARICOCELECTOMY FOR TREATMENT OF CLINICAL VARICOCELES: SHORT-TERM OUTCOMES AND IMPLICATIONS FOR IVF/ICSI**
Matthew Roberts, MD, Ethan Grober, MEd, MD, Kirk Lo, MD, John Beecroft, MD and Keith Jarvi, MD (Presented By: Matthew Roberts)

POSTER 51 **INFLUENCE OF SPERM CRYOPRESERVATION ON THE OOCYTE ACTIVATING FACTOR PLCÆ**
Elke Heytens, John Parrington, PhD, Stijn Lambrecht, PhD, Claire Young, Kevin Coward, PhD, Reza Soleimani, DVM, Jan Gerris, MD, Marc Dhont, MD, Dieter Deforce, PhD and Petra De Sutter, MD (Presented By: Elke Heytens)

POSTER 52 **EFFECT OF THE AGE OF THE MALE ON PREGNANCY RATES ADJUSTED FOR ABNORMAL HYPO-OSMOTIC SWELLING TESTS USING A DONOR EGG MODEL**
Jerome Check, MD, PhD, Kimberly McMonagle, MT, Wendy Hourani, BA and Aniela Bollendorf, MT (Presented By: Jerome Check)

POSTER 53 **EFFECT OF LUBRICANTS DEVELOPED FOR FERTILITY MARKETS ON IN VITRO FERTILIZATION AND EMBRYO DEVELOPMENT**
Raymond W. Wright, Jr., PhD (Presented By: Raymond W. Wright, Jr.)

POSTER 54 **THE EFFECT OF GONADOTROPINS ON BOVINE OOCYTES MATURATION IN VITRO: ANIMAL MODEL**
Huai L. Feng, PhD, Arjun Kadam, PhD, Jeff Pan, MD, Hong Yang, MD, MS, Wegang Zhao, MD and Avner Hershlag, MD (Presented By: Huai L. Feng)

POSTER 55 **THE FREQUENCY OF SUBNORMAL HYPO-OSMOTIC SWELLING TESTS INCREASE WITH ADVANCING AGE OF THE MALE**
Daniel Kramer, Aniela Bollendorf, MT, Jerome Check, MD, PhD and Carrie Wilson, BA (Presented By: Daniel Kramer)

POSTER 56 **THE EFFECT OF THE LACK OF SPERM WITH RAPID LINEAR PROGRESSION ON PREGNANCY RATES FOLLOWING IN VITRO FERTILIZATION WITH INTRACYTOPLASMIC SPERM INJECTION**
Jerome Check, MD, PhD, Wendy Hourani, BA, Danya Horwath, MS, Donna Summers-Chase, MS, Wei Yuan, PhD and Maria Chiara Levito, MS (Presented By: Jerome Check)

POSTER 57 **LOW HYPO-OSMOTIC SWELLING TEST SCORES CORRELATE BETTER WITH LOWER PERCENT MOTILITY THAN ANY OTHER ABNORMAL SEMEN PARAMETERS**
Daniel Kramer, Jerome Check, MD, PhD, Aniela Bollendorf, MT and Wendy Hourani, BA (Presented By: Daniel Kramer)

POSTER 58 **THE MAJORITY OF MALES WITH SUBNORMAL HYPO-OSMOTIC TEST SCORES HAVE NORMAL VIABILITY**
Aniela Bollendorf, MT, Daniel Kramer and Jerome Check, MD, PhD (Presented By: Aniela Bollendorf)

POSTER 59 **TENDENCIES IN THE CLINICAL INDICATIONS OF SPERM DONATION OVER THE LAST 10 YEARS AND ITS EFFECTS ON REPRODUCTIVE OUTCOME**
Marcos Meseguer, PhD, Francisco Minaya, PhD, Antonio Pellicer, MD, Jose Remohí, MD, Manuel Muñoz, MD and Nicolás Garrido, PhD (Presented By: Marcos Meseguer)

POSTER 60 **ABOUT 13% OF WOMEN MAY HAVE THE WRONG METHOD OF OOCYTE INSEMINATION WHEN UNDERGOING IN VITRO FERTILIZATION BY FAILURE TO EVALUATE THE ABNORMAL HYPO-OSMOTIC SWELLING TEST SCORE**
Gabrielle Citrino, Jerome Check, MD, PhD, Aniela Bollendorf, MT, Wendy Hourani, BA and Ann DiAntonio, BS, MT (Presented By: Gabrielle Citrino)

POSTER 61 **EFFECT OF METHOD OF OOCYTE FERTILIZATION ON FERTILIZATION, PREGNANCY AND IMPLANTATION RATES IN WOMEN WITH UNEXPLAINED INFERTILITY**
Aniela Bollendorf, MT, Jerome Check, MD, PhD, Wei Yuan, PhD, Chiara Levito, MS, Kimberly Swenson, MLT and Kimberly McMonagle, MT (Presented By: Aniela Bollendorf)

EPIDIDYMIS / VAS DEFERENS / SEMINAL VESICLES

POSTER 62 **PERK/DUSP6 HAVE REGULATORY ROLES IN ESTABLISHING AND MAINTAINING THE INITIAL SEGMENT OF THE EPIDIDYMIS**
Bingfang Xu, Ling Yang, MD, R. John Lye, PhD and Barry Hinton, PhD (Presented By: Bingfang Xu)

POSTER 63 **VASECTOMY INFLUENCES EXPRESSION OF CRISP1 IN HUMAN EXCURRENT DUCT**
Christine Légare, MSc, Véronique Thimon, PhD, Michel Thabet, MD and Robert Sullivan, PhD (Presented By: Christine Légare)

POSTER 64 **THE IDENTIFICATION OF MOUSE SPERM PROTEINS THAT EXHIBIT DIFFERENTIAL PATTERNING BETWEEN CAPUT AND CAUDA EPIDIDYMAL SPERM**
Takashi Ijiri, PhD, Wenlei Cao, MD and George Gerton, PhD (Presented By: Takashi Ijiri)

POSTER 65 **EXPRESSION OF EPIDERMAL GROWTH FACTOR (EGF) AND ITS RECEPTOR (EGFR) ALONG RAT EPIDIDYMIS AND ISOLATED SPERM**
Marilia Patrao, Acacio Silveira-Neto, Graduated and Maria Christina Avellar, Professor (Presented By: Marilia Patrao)

POSTER 66 **PREPUBERTAL ANTIANDROGEN EXPOSURE: IMPAIRMENT OF THE EPIDIDYMAL FUNCTION IN ADULT RATS**
Juliana Perobelli, BS, Carla Fernandez, MS, Fabiola Toledo, BS, Gary Klinefelter, PhD and Wilma Kempinas, PhD (Presented By: Wilma Kempinas)

POSTER 67 **GLYCOMIC ANALYSIS OF HUMAN SEMINAL PLASMA REVEALS THE PRESENCE OF IMMUNOMODULATORY CARBOHYDRATE FUNCTIONAL GROUPS**
P.C. Pang, B. Tissot, E.Z. Drobnis, H.R. Morris, A. Dell and G.F. Clark (Presented By: E.Z. Drobnis)

POSTER 68 **DOES THE DECREASE IN SPERM P34H CORRELATE WITH THE TIME ELAPSED BETWEEN VASECTOMY AND VASOVASOSTOMY**
WITHDRAWN
Luc Boudreau, Michel-Hugues Lebel, MD, Annie Imbeault, MSc, Christine Légaré, MSc, Robert Sullivan, PhD and Michel Thabet, MD, FRCSC (Presented By: Luc Boudreau)

POSTER 69 **THE ANTI-MICROBIAL BETA-DEFENSIN 23 IS EXPRESSED IN RODENT EPIDIDYMIS AND BINDS TO SPERMATOZOA DURING THEIR EPIDIDYMAL TRANSIT**
Justin Miles, Laura Piehl, Kathy Bowlin and Antoine Makhlouf, MD, PhD (Presented By: Antoine Makhlouf)

poster session 2

Monday, April 6, 2009
11:00 a.m. – 12:30 p.m.
Location: Grand Ballroom, 2nd Floor

MALE SEXUAL FUNCTION

POSTER 70 **DECREASED AKT SIGNALING IN THE CORPORAL TISSUE OF HYPERCHOELSTEROLEMIC APOLIPOPROTEIN E KNOCKOUT MICE**
Donghua Xie, MD, PhD, Lijing Jia, MD, PhD, Feihua Wu, BA and Craig Donatucci, MD (Presented By: Donghua Xie)

POSTER 71 **DECREASED AKT ACTIVATION AND INCREASED PTEN EXPRESSION IN THE CORPORAL TISSUE OF MICE WITH ALLOXAN INDUCED DIABETES**
Donghua Xie, MD, PhD, Lijing Jia, MD, PhD, Feihua Wu, BA and Craig Donatucci, MD (Presented By: Donghua Xie)

POSTER 72 **MATHEMATICAL VALIDATION OF DUPLEX DOPPLER ULTRASOUND DATA TO PREDICT PENILE HEMODYNAMICS IN MEN WITH ERECTILE DYSFUNCTION**
Suresh Sikka, PhD, Gurdial Arora, PhD, Tumulesh Solanky, PhD and Wayne J.G. Hellstrom, MD (Presented By: Suresh Sikka)

POSTER 73 **ENHANCED ALPHA 1-ADRENOCEPTOR AND P2X-PURINOCEPTOR MEDIATED RESPONSIVENESS IN VAS DEFERENS OF NITRIC OXIDE-DEFICIENT HYPERTENSIVE RATS: PARTIAL PROTECTION BY SILDENAFIL**
Serap Gur, PhD, Suresh Sikka, PhD and Wayne Hellstrom, MD (Presented By: Serap Gur)

POSTER 74 **ACUTE CHANGES IN PENILE OXIMETRY AFTER SINGLE UTILIZATION OF THE VACUUM ERECTION DEVICE (VED) COMPARED TO INTRAURETHRAL ALPROSTATDIL (IUA) AND INTRCAVERNOSAL INJECTIONS (ICI) IN PATIENTS FOLLOWING RADICAL PROSTATECTOMY (RP)**
Artrit Bytyci, BS, Brianne Goodwin, BS, Joseph Alukal, MD and Andrew McCullough, MD (Presented By: Artrit Bytyci)

POSTER 75 **THE CLINICAL ASSOCIATION BETWEEN FREE ANDROGEN INDEX AND ERECTILE DYSFUNCTION IN MEN FROM THE MIDDLE EAST**
Ali Thwaini, A. Hameed, M.Z. Aslam, I. Shergill, Raed Ahmed, Gada Yahia, Donald Morgan and Frank Chinegwundoh
(Presented By: Ali Thwaini)

POSTER 76 **PARTIAL PENILE SENSORY AXON RESECTION (PPSAR) FOR MANAGEMENT OF EJACULATION PRAECOX**
Aref Elseweifi, consultant of urology (Presented By: Aref Elseweifi)

POSTER 77 **MPACT OF LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION ON SEX HORMONES IN HYPOGONADAL MEN**
Wayne Hellstrom, MD, Leonard Marks, MD, Ronald Swerdloff, MD, Christina Wang, MD, Joel Kaufman, MD, Evan Goldfischer, MD, Martin Miner, MD and Ridwan Shabsigh, MD (Presented By: Joel Kaufman)

POSTER 78 **IMPROVED MALE SEXUAL FUNCTION WITH LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION**
Wayne Hellstrom, MD, Leonard Marks, MD, Ronald Swerdloff, MD, Christina Wang, MD, Joel Kaufman, MD, Evan Goldfischer, MD, Martin Miner, MD and Ridwan Shabsigh, MD (Presented By: Wayne Hellstrom)

POSTER 79 **EFFECTS OF LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION ON PROSTATE HEALTH OUTCOMES IN HYPOGONADAL MEN**
Wayne Hellstrom, MD, Leonard Marks, MD, Ronald Swerdloff, MD, Christina Wang, MD, Joel Kaufman, MD, Evan Goldfischer, MD, Martin Miner, MD and Ridwan Shabsigh, MD (Presented By: Joel Kaufman)

POSTER 80 **TIME DEPENDENT CHANGES IN PENILE OXIMETRY AFTER SINGLE APPLICATION OF VACUUM ERECTION DEVICE (VED) IN MEN AFTER RADICAL PROSTATECTOMY (RP)**
Patrick Mufarrij, MD, Artrit Bytyci, BS, Brianne Goodwin, BS, Joseph Alukal, MD and Andrew McCullough, MD (Presented By: Patrick Mufarrij)

POSTER 81 **IMPROVED DEPRESSION-RELATED OUTCOMES IN HYPOGONADAL MEN TREATED WITH LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION**
Wayne Hellstrom, MD, Leonard Marks, MD, Ronald Swerdloff, MD, Christina Wang, MD, Joel Kaufman, MD, Evan Goldfischer, MD, Martin Miner, MD and Ridwan Shabsigh, MD (Presented By: Wayne Hellstrom)

POSTER 82 **PENILE AUGMENTATION SURGERY USING AUTOGRAFT, ALLOGRAFT OR XENOGRAFT**
Joon Yong Kim, MD, Byung Moo Philip Kim, MR, Si Jin Paul Kim Kim, MR, Hee Kyung Kim, professor and Beom Joon Kim, professor (Presented By: Joon Yong Kim)

POSTER 83 **PARTIAL PENILE AUGMENTATION SURGERY WITH MULTIPLE PUNTURE SLING METHOD**
Joon Yong Kim, MD, Byung Moo Philip Kim, MR, Si Jin Paul Kim, MR and Beom Joon Kim, professor (Presented By: Joon Yong Kim)

ANDROGENS/ENDOCRINOLOGY

POSTER 84 **LIQUID CHROMATOGRAPHY-MASS SPECTROMETRIC ASSAY OF TESTOSTERONE AND CORTISOL IN SALIVARY FOR DIAGNOSING MALE ANDROPAUSE**
Eitetsu Koh, PhD, MD, Futoshi Matsui, PhD, MD, Kazuhiro Sugimoto, MD, Hosu Sin, MS, Yuji Maeda, PhD, MD and Mikio Namiki, PhD, MD (Presented By: Eitetsu Koh)

poster session 2

POSTER 85 **INDUCTION OF SPERMATOGENESIS AND FERTILITY DURING GONADOTROPIN TREATMENT OF GONADOTROPIN DEFICIENT INFERTILE MEN: CLUES TO IMPROVING FERTILITY OUTCOME**
Peter Liu, MBBS, FRACP, PhD, H.W. Gordon Baker, MBBS, FRACP, PhD, Veena Jayadev, MBBS, FRACP, Margaret Zacharin, MBBS, FRACP, Ann Conway, MBBS, FRACP and David Handelsman, MBBS, FRACP, PhD (Presented By: Peter Liu)

POSTER 86 **SERUM INHIBIN B, BUT NOT FSH, LEVELS ARE SIGNIFICANTLY ALTERED IN OBESE MALES**
Tyl Taylor, BS, MS, Micheal Glasner, MD and William Roudebush, BS, MS, PhD (Presented By: Tyl Taylor)

POSTER 87 **AN OPEN LABEL PHASE III STUDY OF FORTIGEL™ (TESTOSTERONE) 2% GEL IN HYPOGONADAL MALES**
Adrian Dobs, MD, John McGettigan, MD, Mark Akerson, MD, Paul Norwood, MD, Julian Howell, MB, BS and Elizabeth Waldie, BSc (Hons) (Presented By: Adrian Dobs)

POSTER 88 **LEYDIG CELL SPECIFIC CONDITIONAL DELETION OF ALK3**
Xiufeng Wu, Ningning Zhang, MS and Mary M. Lee, MD (Presented By: Xiufeng Wu)

POSTER 89 **TESTOSTERONE ADMINISTRATION TO ELDERLY HYPOGONADAL IMPROVES THE METABOLIC SYNDROME, C-REACTIVE PROTEIN AND LIVER STEATOSIS**
Farid Saad, PhD[1] and Ahmad Haider, MD[2] (Presented By: Farid Saad)

POSTER 90 **CYP2A1 INTERACTS WITH 11Â-HYDROXYSTEROID DEHYDROGENASE 1 IN RAT LEYDIG CELLS**
Ping Wang, Guo-Xin Hu, Hong-Yu Zhou, Bing-Bing Chen, Qing-Quan Lian, Kun-Ming Chen, Dianne O. Hardy, Pramod Kumar, Narender Kumar, Quang Liang, Xiao-kun Li and Ren-Shan Ge (Presented By: Ping Wang)

POSTER 91 **FGF2 AND LH AFFECT ANDROGEN PRODUCTION IN RAT IMMATURE LEYDIG CELLS VIA DIFFERENT INTRACELLULAR PATHWAY**
Yechen Xiao, PhD, Xiaokun Li, MD, Huiyan Wang, MD, Dianne O. Hardy, PhD and Renshan Ge, MD (Presented By: Yechen Xiao)

POSTER 92 **THE (+)- AND (-)-GOSSYPOLS POTENTLY INHIBIT HUMAN AND RAT 11Â-HYDROXYSTEROID DEHYDROGENASE TYPE 2**
Bingbing Chen, Han Lin, PhD, Guo-Xin Hu, MD, Ying Su, MD, Hong-Yu Zhou, MS, Qing-Quan Lian, PhD, Hui Cai, MD, Dianne O. Hardy, PhD, Ding-Ying Gu, MD and Ren-Shan Ge, MD (Presented By: Bingbing Chen)

POSTER 93 **THE (+)-AND (-)-GOSSYPOLS POTENTLY INHIBIT BOTH 3Â-HYDROXYSTEROID DEHYDROGENASE AND 17Â-HYDROXYSTEROID DEHYDROGENASE 3 IN HUMAN AND RAT TESTES**
Hong-Yu Zhou, Master, Guo-Xin Hu, Bachelor, Xing-Wang Li, Doctor, Bing-Bing Chen, Master, Ye-Chen Xiao, Doctor, Qing-Quan Lian, Bachelor, Guang Liang, Doctor, Howard H. Kim, Doctor, Xiao-Kun Li, Doctor, Dianne O. Hardy, Doctor and Ren-Shan Ge, Master (Presented By: Hong-Yu Zhou)

POSTER 94 **ANDROGENIC ACTIVITY OF ALLIUM CEPA ON SPERMATOGENESIS IN RAT**
Arash Khaki, DVM, PhD (Presented By: Arash Khaki)

POSTER 95 **SAFETY STUDY OF LONG-ACTING PARENTERAL TESTOSTERONE OVER 24-30 MONTHS**
Farid Saad, PhD and Ahmad Haider, MD (Presented By: Farid Saad)

POSTER 96 **STUDY OF THE CYCLICAL CHANGES OF REPRODUCTIVE HORMONES IN SERUM AFTER EJACULATION IN NORMAL ADULT MEN**
Kangshou Yao and Junrong Zhang (Presented By: Kangshou Yao)

SPERMATOGENESIS / STEROIDOGENESIS / TESTIS BIOLOGY

POSTER 97 **PROTEOMIC ANALYSIS OF PROTEINS INVOLVING IN SPERMIOGENESIS IN MOUSE**
Xuejiang Guo, PhD candidate, Jian Shen, PhD candidate, Ping Zhang, PhD candidate, Rui Zhang, PhD candidate, Chun Zhao, PhD candidate, Jun Xing, PhD candidate, Ling Chen, master candidate, Min Lin, bachelor, Zuomin Zhou, PhD, Bin Su, PhD and Jiahao Sha, PhD (Presented By: Xuejiang Guo)

POSTER 98 **CHRONIC FETAL / POSTNATAL HYPOTHYROIDISM TRANSIENTLY INHIBITS SERTOLI CELL ANDROGEN RECEPTOR EXPRESSION AND CONSEQUENTLY CAUSES A DELAY IN SPERMATOGENESIS**
Katja Teerds, PhD, Dirk de Rooij, PhD, Hans Swarts, Anita van Kesteren-Buiting, Anne Klimstra, BSc, Jaap Keijer, PhD and Eddy Rijntjes, PhD (Presented By: Katja Teerds)

POSTER 99 **DEVELOPMENTALLY REGULATED ACTIVIN A SIGNAL TRANSDUCTION BY SERTOLI CELLS IS REQUIRED FOR NORMAL MOUSE TESTIS DEVELOPMENT**
Catherine Itman, PhD, Chris Small, PhD, Michael Griswold, PhD, Ankur K Nagaraja, Martin Matzuk, PhD, Chester Brown, PhD, Matthias Ernst, PhD, David A. Jans, PhD and Kate L. Loveland, PhD (Presented By: Catherine Itman)

POSTER 100 **SYNAPTIC DISRUPTIONS, ABERRANT SUMO-1 ASSOCIATION, AND UBIQUITINATION OF ZYGOTENE-PACHYTENE SPERMATOCYTES IN A MAN WITH MEIOTIC MATURATION ARREST: A CASE STUDY**
Petrice Brown, MA, PhD, Peter Schlegel, MD and Patricia Morris, PhD (Presented By: Petrice Brown)

POSTER 101 **DIRECT INTERACTIONS BETWEEN INSULIN-LIKE GROWTH FACTOR BINGDING PROTEIN-3 AND BAX PROMOTES MALE GERM CELL APOPTOSIS IN RAT**
Yue Jia, MD, PhD, Yanhe Lue, Amiya P. Sinha Hikim, Laura J. Cobb, David Hwang, Vince Atienza, Pinchas Cohen, Ronald S. Swerdloff and Christina Wang (Presented By: Yue Jia)

poster session 2

POSTER 102 **XENOGRAFTING PROVIDES EVIDENCE FORA ROLE OF PARACRINE FACTORS IN THE PATHOPHYSIOLOGY OF EQUINE IDIOPATHIC TESTICULAR DEGENERATION**
Regina Turner, VMD, PhD, Wenxian Zeng, PhD, Mark Modelski and Ina Dobrinski, Drmedvet, MVSc, PhD (Presented By: Regina Turner)

POSTER 103 **ACTIVIN SIGNALLING MODULATORS IN NORMAL, GONADOTROPIN-DEPRIVED, AND NEOPLASTIC HUMAN TESTIS**
Vinali Dias, Ewa Rajpert-De Meyts, Mai A. Sarraj, Rob McLachlan and Kate L. Loveland (Presented By: Vinali Dias)

POSTER 104 **PROFILING MICRORNAS IN INFERTILE MEN WITH SERTOLI CELL-ONLY SYNDROME**
Chia Ling Chung, MSc (Presented By: Chia Ling Chung)

POSTER 105 **LOCALIZATION OF TOPOISOMERASE IIÂ IN RAT GERM CELLS DURING SPERMIOGENESIS**
Lihua Liu, MD, Benjamin R. Emery, MPhil and Douglas T. Carrell, PhD (Presented By: Lihua Liu)

ENVIRONMENT/TOXICOLOGY

POSTER 106 **EFFECTS OF PBDES ON ANDROGEN PRODUCTION OF ADULT LEYDIG CELLS**
Yunhui Zhang, PhD, Chantal Sottas, MD and Renshan Ge, PhD (Presented By: Yunhui Zhang)

POSTER 107 **EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE ON RAT LEYDIG CELL REGENERATION**
Xingwang Li, PhD, MD, Qing-Quan Lian, PhD, MD and Ren-Shan Ge, MD (Presented By: Xingwang Li)

POSTER 108 **COMPOUNDING EFFECTS OF LEAD and NICOTINE ON RAT SPERM DNA DAMAGE IN A SURGICALLY INDUCED VARICOCELE MODEL**
Matthew Sanderson, BS, Benjamin R. Emery, MPhil, Yong En Sun, MD, C. Matthew Peterson, MD and Douglas T. Carrell, PhD (Presented By: Matthew Sanderson)

PROSTATE/TESTIS CANCER/CLINICAL UROLOGY

POSTER 109 **REAL-TIME PERIPROSTATIC TISSUE IMAGING WITH MULTIPHOTON MICROSCOPY FOR IMPROVING POTENCY OUTCOMES DURING NERVE-SPARING RADICAL PROSTATECTOMY: INITIAL RESULTS FROM FRESH HUMAN PROSTATECTOMY SPECIMENS**
Gerald Tan, MB, ChB, MRCSEd, MMed, FAMS, Rajiv Yadav, MD, Michael Herman, MD, Sushmita Mukherjee, PhD, Frederick Maxfield, PhD, Watt Webb, DSc and Ashutosh Tewari, MD, MCh (Presented By: Gerald Tan)

POSTER 110 **THE NATURAL HISTORY OF SMALL INCIDENTAL HYPOECHOIC TESTICULAR MASSES IN INFERTILE MEN: IS SURVEILLANCE THE NEW STANDARD OF CARE?**
Matthew Roberts, MD, Paul Toren, MD, Irene Lecker, Ethan Grober, MEd, MD, Keith Jarvi, MD and Kirk Lo, MD (Presented By: Matthew Roberts)

POSTER 111 **MOLECULAR INTERACTIONS BETWEEN GALECTIN-3 AND PROSTATE SPECIFIC ANTIGEN (PSA) SECRETED BY PROSTATE CANCER CELL LINES**
Rebecca Gilbride, BS, Ashley Block, BS, Sarika Saraswati, BS, MS, PhD and Alan Diekman, BS, PhD (Presented By: Rebecca Gilbride)

POSTER 112 **RELATIONSHIP BETWEEN NON-ENZYMIC ANTIOXIDANT PROFILE AND MEAN PROSTATE SPECIFIC ANTIGEN (MPSA) LEVELS OF KNOWN PROSTATE CANCER PATIENTS**
Oluyemi Akinloye, PhD, Olatunji I. Kareem, FMLSCN and Oluwatosin A. Adaramoye, PhD (Presented By: Oluyemi Akinloye)

POSTER 113 **OLD MARKERS WITH NEW MEANINGS FOR PROSTATE CANCER**
Xiao Gu, MD, PhD, Jin Yang, MD, Hong Zhao, MD, PhD and Tao Gu, MD, PhD (Presented By: Xiao Gu)

GENETICS

POSTER 114 **A NOVEL MOUSE MODEL OF MALE INFERTILITY – A LINK TO LUNG CANCER**
Duangporn Jamsai, PhD, Stephanie Smith, BSc (Hons), Claire Borg, PhD, Victoria Adams, BSc (Hons), Donna J. Merriner, BSc (Hons) and Moira K. O'Bryan, PhD (Presented By: Duangporn Jamsai)

POSTER 115 **REACTIVE OXYGEN SPECIES AND SPERM MITOCHONDRIAL DNA MUTATIONS IN INFERTILE PATIENTS**
Sundararajan Venkatesh, MPharm, Rakesh Kumar, MSc, Monis Shamsi, MSc, Sankalp Dudeja, MBBS student, Rajeev Kumar, MD, Narmada Gupta, MD and Rima Dada, MD PhD (Presented By: Sundararajan Venkatesh)

POSTER 116 **DYNAMICS OF PRE ZYGOTIC AND POST ZYGOTIC SEX RATIO**
Ashutosh Halder, MD, DNB, DM (Presented By: Ashutosh Halder)

POSTER 117 **MITOCHONDRIAL DNA MUTATION ANALYSIS IN SEMEN AND BLOOD SAMPLES OF INFERTILE OLIGOASTHENOZOOSPERMIC (OA) MEN**
Sundararajan Venkatesh, MPharm, Rakesh Kumar, MSc, Monis Shamsi, MSc, Sankalp Dudeja, MBBS student, Rajeev Kumar, MD, Narmada Gupta, MD and Rima Dada, MD, PhD (Presented By: Sundararajan Venkatesh)

poster session 2

OTHER

POSTER 118 **ANTIMICROBIAL ACTIVITY OF THE CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PROTEIN**
Qing Yuan, MD, Chen Xu, MD, Yanqin Hu and Yifei Wang (Presented By: Qing Yuan)

POSTER 119 **LIPID PEROXIDATION IN TESTIS AND EPIDIDYMIS UNDER INTERMITTENT HYPOBARIC HYPOXIA: PROTECTIVE ROLE OF ASCORBIC ACID**
Jorge Farias, PhD, Mariela Puebla, biochemist, Alejandro Acevedo, student, Pablo Tapia, biochemist, Eduardo Gutierrez, student, Andrea Zepeda, student, Camila Juantok, student, Gloria Calaf, PhD and Juan Reyes, PhD (Presented By: Jorge Farias)

POSTER 120 **A NOVEL AND MICROWOUND SURGICAL PROCEDURE FOR CIRCUMCISION USING THE CHINA SHANG RING**
Chen Bin, Cheng Yue, MD, Peng Yifeng, MD, Liu Yangdong, MD, Tian Long, MD and Lu Nianqing, MD (Presented By: Chen Bin)

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Sunday, April 5, 2009
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session I
Basic Research
Moderators: Barry Hinton, PhD
Sophie-Ann Lamour, BSc
Location: Columbus Ballroom, 2nd Floor

1

IGFBP-3 ENHANCES GERM CELL APOPTOSIS INDUCED BY TESTICULAR HORMONAL DEPRIVATION

YanHe Lue, MD[1], Hemal Mehta, PhD[2], QingHai Liu, MD[1], Pinchas Cohen, MD[2], Yue Jia, PhD[1], Amiya Sinha Hikim, PhD[1], Ronald Swerdloff, MD[1] and Christina Wang, MD[1] (Presented By: YanHe Lue)
[1]Division of Endocrinology, Department of Medicine, LABioMed at Harbor-UCLA Medical Center, Torrance, CA; [2]Pediatric Endocrinology, Mattel Children's Hospital, David Geffen School of Medicine at UCLA, Los Angeles, CA

Germ cell apoptosis is one of the major mechanisms of suppression of spermatogenesis induced by intratesticular deprivation of testosterone. Gene microarray analysis showed that insulin-like growth factor binding protein-3 (IGFBP-3) was significantly up regulated (2.7-fold) by treatment with testosterone alone, or in combination with Levonorgestrel, in human testes. To study the action of IGFBP-3 in male germ cell apoptosis, we first characterized the testicular phenotype of adult IGFBP-3 knockout mice. We then treated 5 adult (14-15 week-old) IGFBP-3 knockout and 5 age-matched wild-type mice with Acyline (GnRH-A, 20mg/kg BW) for 2 weeks to induce germ cell apoptosis. The results showed that there were no differences in testis weight (TW) and the rate of germ cell apoptosis between wild-type (TW: 86.8±13.2mg) and IGFBP-3 knockout (TW: 96.3±2.3mg) mice. GnRH-A treatment significantly decreased TW of wild-type mice (TW: 80.6±3.7mg), but not of IGFBP-3 knockout (TW: 95±5.2mg) mice. GnRH-A treatment significantly increased the incidence of germ cell apoptosis (apoptotic germ cells/100 Sertoli cells) in wild-type mice at stages VII-VIII (51.5±11.3) and XI-XII (53.6±5.5) as compared to age-matched IGFBP-3 knockout mice (VII-VIII: 9.5±1.2; XI-XII: 26±2.8). To further investigate the effect of IGFBP-3 on spermatogenesis, groups of 4 adult rats (60-day-old) received one of the following treatments for 5 days: 1) daily intratesticular injections of saline (controls) or IGFBP-3 (50 mcg), 2) a single sc injection of GnRH-A, acyline (30mg/Kg BW), and 3) GnRH-A injection on day 1 and daily intratesticular injection of 50 mcg IGFBP-3. Intratesticular administration of IGFBP-3 alone in rats significantly increased germ cell apoptosis at stages VII-VIII (8.9±0.6) and XIV-I (46.5±9.2) as compared to control (VII-VIII: 2.1±0.3; XIV-I: 12.1±1.2). As expected, GnRH-A treatment increased germ cell apoptosis at stages VII-VIII (38.4±4.8) and XIV-I (21.1±1.0). Addition of IGFBP-3 to GnRH-A treatment further increased germ cell apoptosis at stages VII-VIII (82.5±6.3) than either treatment alone. We conclude that 1) testicular hormonal deprivation induced germ cell apoptosis is partially mediated through IGFBP-3 action; 2) administration of IGFBP-3 induces apoptosis, and further enhances hormone deprivation-induced germ cell apoptosis in testes. These findings may have implications for male fertility and testicular disease.

2

EFFECTS OF DI- (2-ETHYLHEXYL) PHTHALATE ON RAT LEYDIG CELL REGENERATION

Xingwang Li, PhD, MD, Qing-Quan Lian, PhD, MD and Ren-Shan Ge, MD (Presented By: Xingwang Li)
Population Council and Rockefeller University

Background and Objectives: Di-(2-ethylhexyl) phthalate (DEHP), the most widely used plasticizer, has developmental and reproductive toxicity. It has been demonstrated that pubertal DEHP exposures increased Leydig cell number but decreased steroidogenesis. However, whether the increased number is caused by the increases of proliferation of stem Leydig cell (SLC) and its commitment into progenitor Leydig cell (PLC) is unclear. The goal of the present study is to determine whether there is increase of proliferation of SLC and its commitment into PLC and thereafter long-term effects on the Leydig cell regeneration.
Methods: 90-day-old Long-Evans rats were randomly divided into 3 groups, and were gavaged with the corn oil vehicle or DEHP 10 or 750 mg/kg daily for 42 days. All rats received intraperitoneal (i.p.) injection of 75 mg/kg body weight (BW) ethane dimethanesulfonate (EDS) to kill adult Leydig cells (ALCs) 7 days after DEHP treatment. The rats were then killed 11, 21, 28 and 42 days after DEHP treatment. One hour before being killed, the rats were received i.p. injection of 5-bromo-29-deoxyuridine (BrdU) at 100 mg/kg BW. Serum testosterone (T) levels were assessed by RIA. The mRNA expression levels of Leydig cell genes were measured by real-time PCR.
Results: EDS killed ALCs 4 days after EDS treatment as judged by undetectable serum T and undetectable 3â-hydroxysteroid dehydrogenase (3â-HSD) positive cells in the interstitium in control testis. However, in both DEHP treatment groups, there were detectable serum T and some spindle-shaped 3â-HSD positive cells in the interstitium. These 3â-HSD positive cells were not stained by the antibody against 11â-hydroxysteroid dehydrogenase 1 (11â-HSD1), a marker for immature and adult Leydig cells. Real-time PCR detected the disappearance of Leydig cell marker mRNA levels including Lhcgr, Cyp11a1, Cyp17a, Insl3 (ALC marker) and Hsd11b1 in control testes. However, there were detectable levels of Lhcgr, Cyp11a1 and Cyp17a mRNA and undetectable levels of Insl3 and Hsd11b1 in DEHP groups, indicating that these 3â-HSD positive cells were PLCs. The mRNA levels for Nes (marker for SLC) significantly increased in control testes, but not in DEHP treated testes, suggesting that few SLCs were present in DEHP treated testes.
Conclusion: The present study suggests that DEHP stimulates the proliferation of SLC and their commitment to PLCs.

3

ABSENCE OF ESTROGEN RECEPTOR ALPHA LEADS TO A MISREGULATION OF THE EPIDIDYMAL FLUID MILIEU AND A CONSEQUENT DEFECT IN SPERM FUNCTION

Avenel Joseph, Rex Hess, PhD[1], Chemyong Ko, PhD[2] and Barry Shur, PhD[3] (Presented By: Avenel Joseph)[1]University of Illinois Urbana-Champaign Department of Veterinary Biosciences; [2]University of Kentucky Center of Excellence in Reproductive Sciences; [3]Emory University, Department of Cell BiologyEstrogen receptor-alpha (ERá) is highly expressed in the efferent ductules and epididymal epithelium and is essential for the normal function of the male reproductive tract. Male mice deficient in ERá (ERáKO mice) are infertile and sperm recovered from the cauda epididymis exhibit reduced motility and fail to fertilize eggs in vitro. These effects on sperm appear to result from defective epididymal function and not a direct effect on spermatogenesis, as ERáKO germ cells transplanted into wild-type testes yield normal offspring. It has been shown previously that the absence of a functional ERá leads to transcriptional down-regulation of Na(+)/H(+) exchanger-3 (NHE3) and reduced fluid reabsorption in efferent ductules. We hypothesized that this reduction in water and ion transport would lead to alterations in the epididymal fluid milieu, which would negatively impact sperm physiology. Analysis of the epididymal fluid revealed that the ERáKO maintains a higher luminal pH throughout the epididymis, confirming an inability of the efferent ducts and/or epididymis to properly acidify the luminal contents. Similarly, the osmolality of the ERáKO epididymal fluid is reduced relative to wild-type, confirming inappropriate water absorption. Subsequent studies showed that these abnormalities were not the result of global defects in epididymal function, since protein secretion by the ERáKO epididymis appeared normal as judged by SDS-PAGE of total secreted proteins and by immunoblotting of candidate secreted proteins. To gain insight into the basis of the aberrant fluid homeostasis in the ERáKO epididymis, the expression of several enzymes and transporters known to be involved in acid/base regulation was analyzed. The levels of NHE3, as well as carbonic anhydrases II and XIV, were all reduced in the proximal portion of the ERáKO epididymis, while other components appeared unaffected, including other carbonic anhydrases and V-ATPase. To determine the effect of the altered epididymal milieu on ERáKO sperm, the internal sperm pH was assayed and shown to be markedly elevated in ERáKO sperm relative to wild-type. Furthermore, ERáKO sperm recovered from the epididymis were found to have abnormal axoneme coiling and increased incidence of spontaneous acrosome reactions, both of which are consistent with defective regulation of the epididymal fluid. Additional studies will further examine the mechanisms by which estrogen influences sperm function and fertilizing ability.

4

A NOVEL MOUSE MODEL OF ASTHENOSPERMIA

Claire Borg[1], Victoria Adams[2], David de Kretser[1] and Moira O'Bryan[1] (Presented By: Claire Borg)
[1]Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia; [2]Australian Phenomics Facility, Australian National University, Canberra, Australian Capital Territory, Australia

The Mot1 mouse line was identified in a forward genetics (phenotype to genotype) ENU mutagenesis screen for infertile mouse lines. This mouse line was identified as having male-specific infertility. Using natural matings, Mot1 males are sterile. Sperm were also unable to achieve fertilization via in vitro fertilization. The testes of Mot1 mice display qualitatively normal spermatogenesis at a histological level, however, calculation of daily sperm production shows a 50% reduction compared to fertile Mot1 testes. Computer assisted sperm analysis (CASA) showed that both total sperm motility and progressive motility were significantly reduced when compared to fertile littermates.
The causal mutation was recently identified by using the Affymetrix GeneChip Mouse Mapping 5K SNP kit and subsequent fine mapping and candidate gene sequencing. A point mutation that was homozygous in all infertile Mot1 mice and either heterozygous or wild type in fertile littermates was identified in a previously uncharacterized gene that contains protein domains suggesting an involvement in GTP-binding. GTP-binding is important in intracellular processes such as cytoskeletal organization and protein transport and as such, a breakdown in its normal function could result in abnormal axonemal formation or disrupted intraflagellar transport of proteins which is essential to sperm development and function, thus causing the observed infertility. The A to G transition in the cDNA of the Mot1 gene results in an amino acid change from an acidic and polar aspartic acid to a neutral, nonpolar glycine. This gene is expressed in a wide range of tissues, and appears to be enriched in tissues that are ciliated and thus the presence of other ciliopathies is currently being investigated.
The identification of a mutation causing the Mot1 phenotype has identified a novel regulator of male fertility that is required for sperm development, axoneme function and motility.

5

JAM-A INTERACTS WITH CASK AND PMCA4 IN THE PLASMA MEMBRANE AND REGULATES CA2+ HOMEOSTASIS AND MURINE SPERM MOTILITY

RolandsAravindan, PhD, Ulhas Naik, PhD, Victor Fomin, PhD, Randall Duncan, PhD, Deni Galileo, PhD and Patricia Martin-DeLeon, PhD (Presented By: Rolands Aravindan)
Department of Biological Sciences, University of Delaware

Previously we reported that deletion of murine Junctional adhesion molecule-A (Jam-A) results in reduced sperm motility and an abnormal mitochondrial morphology reminiscent of the Ca2+ overload seen in Plasma membrane calcium/calmodulin-dependentATPase 4 (PMCA4) null mice. Here we show that steady-state intracellular Ca2+ concentrations [Ca2+]i in capacitated and uncapacitated Jam-A null sperm, as detected by spectrofluorometry, are significantly higher ($P<0.001$) than in wild-type (WT). Confocal microscopy revealed Ca2+ accumulation over the acrosome and on the midpiece of the flagellum. In the presence of carbonyl cyanide m-chlorophenylhydrazone (CCCP), an uncoupler of mitochondrial oxidative phosphorylation, Ca2+ levels in null sperm spiked significantly higher than WT ($P<0.001$), indicating a disproportionate release of Ca2+ from mitochondrial stores. Despite greater mitochondrial sequestration of Ca2+ in Jam-A null sperm, significantly higher numbers compared to WT ($P<0.001$) exhibited Rhodamine 123 uptake, reflecting their normal mitochondrial potential. However, ATP concentrations in capacitated and uncapacitated Jam-A null sperm were significantly ($P<0.001$) lower than controls. This explains their reduced motility. Although CCCP elicited the expected decrease in ATP in capacitated WT sperm (-31.3%, $P<0.001$), Jam-A null sperm showed a significant increase (+17.1%, $P<0.01$) likely due to an enhancement of glycolysis in the presence of elevated [Ca2+]i. Since glycolysis and mitochondrial potential are not adversely affected in nulls, the motility defect is likely not due to reduced ATP production. For reasons stated below we suggest that the defective motility in JAM-A nulls results from increased ATP consumption by the Ca2+ efflux pump, PMCA4. We have identified that: 1) in null sperm CASK (Calcium/calmodulin dependent serine kinase), a scaffolding membrane protein, is ~6-fold overexpressed, 2) CASK and JAM-A co-localize on the principal piece of the sperm flagellum and also co-immunoprecipitate indicating that they are interacting partners, and 3) PMCA4 co-localizes and co-immunoprecipitates with CASK. Thus these three proteins are intimately associated in the plasma membrane, possibly via their mutual PDZ-domain and domain-binding sequences. These novel findings reveal a mechanism of the regulation of the Ca2+ efflux pump in sperm and the role of JAM-A in Ca2+ homeostasis. (NIH COBRE grant # 5P20RR015588-08)

6

DNA BINDING ACTIVITY OF HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) IN THE NORMOXIC AND ISCHEMIC TESTIS

Anoop Shah, Rebecca Tyson and Michael Palladino, BS, PhD (Presented By: Anoop Shah)
Monmouth University

Spermatic cord torsion can lead to testis ischemia (I) and subsequent ischemia-reperfusion (I/R) resulting in germ cell specific apoptosis yet Leydig cells are not susceptible to torsion-induced apoptosis. Previously we demonstrated that the transcription factor hypoxia-inducible factor-1 (HIF-1) is abundant in Leydig cells. HIF-1 is a heterodimer of á and â subunits and a key regulator of physiological responses to hypoxia in cancer, cardiac ischemia, stroke and many other conditions. In most tissues HIF-1á is degraded under normoxic conditions and stabilized by hypoxia while HIF-1â is constitutively produced. In the rat, HIF-1 subunits are abundant in both the normoxic and hypoxic testis. We hypothesize that HIF-1 and Leydig cells are important for regulating oxygen microenvironments in the testis and that HIF-1 may activate expression of antiapoptotic target genes to protect Leydig cells from apoptosis following I and I/R. The purpose of this study was to determine if HIF-1 is active in the normoxic and ischemic testis and to identify target genes for testicular HIF-1. Unilateral testicular torsion (720 degrees) in adult Sprague-Dawley rats was surgically induced for 1h of I or 1/4h of I/R. Cytoplasmic and nuclear proteins were extracted from sham and torsed testes and an Active Motif TransAM™ enzyme-linked immunosorbent (ELISA) assay used to quantitate HIF-1 binding to a consensus hypoxia response element (5'-RCGTG-3') from the human erythropoietin gene promoter. Nuclear protein extracts were also used for chromatin immunoprecipitation (ChIP) analysis to identify HIF-1 target genes. Testicular HIF-1 from normoxic and ischemic testes displayed equivalent DNA binding activity by the HIF ELISA assay, suggesting that HIF-1 is an active transcription factor in both the normoxic and ischemic testis. ChIP analysis identified enrichment of promoter sequences for the gene induced myeloid leukemia cell differentiation 1 (Mcl-1). Mcl-1 protein is abundant in both normal and ischemic testes. In a pattern identical to HIF-1, steady-state levels of Mcl-1 protein were not significantly affected ($p<0.05$) by I or I/R. Mcl-1 also co-localizes with HIF-1á in Leydig cells. In conclusion, results demonstrate that HIF-1 displays DNA binding activity in both the normoxic and ischemic testis and suggest that Mcl-1 may be a key target gene for testicular HIF-1 with potentially important roles in antiapoptotic protection of Leydig cells. Supported by NIH R15HD046451.

Sunday, April 5, 2009
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session II
Clinical Research
Moderators: Robert Oates, MD
Ajay Nangia, MBBS
Location: Washington Room, 3rd Floor

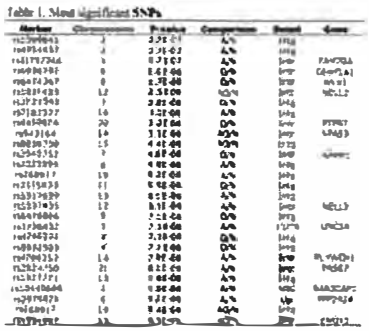
7

GENOME-WIDE SCAN FOR SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ASSOCIATED WITH SEVERE OLIGOZOOSPERMIA AND AZOOSPERMIA IN MEN

Kenneth Aston, PhD and Douglas Carrell, PhD (Presented By: Kenneth Aston)
University of Utah Andrology and IVF Laboratories

Objective: A number of small-scale, sequencing studies seeking to identify SNPs associated with male infertility have yielded few promising results. This pilot study employed whole genome microarray technology to scan over 370,000 genetic loci in 240 individuals to identify SNPs associated with severe oligozoospermia (oligo) and azoospermia (azoo) in men.

Methods: DNA from normospermic controls (n=101), oligo (<5x106 sperm/ml; n=64), and azoo men (n= 75) was delivered to deCODE Genetics for hybridization and genotyping using Illumina 370K-duo BeadChips. Genotypes were analyzed for associations using HelixTree Software. Associations were tested based on the following comparisons: azoo + oligo vs. control, azoo vs. control, and oligo vs. control.
Results: A total of 26 markers not previously identified to be associated with male infertility were significantly different between groups at p <1x10-5 (Table 1). Significant markers include 13 intergenic SNPs, 10 intronic SNPs, one SNP located proximally upstream of a gene, one SNP in a 3' untranslated region (3'UTR), and one non-synonymous coding SNP.
Conclusion: This study, representing the first genome-wide scan for male infertility-associated SNPs, has identified a number of potentially important loci. Follow-up studies involving additional cases and controls must be performed to validate these findings. In addition, follow-up studies evaluating gene and protein expression and function will provide further insight into the genetic causes of male infertility.



8

RACIAL DIFFERENCES IN VASECTOMY UTILIZATION IN THE UNITED STATES: DATA FROM THE NATIONAL SURVEY OF FAMILY GROWTH

Michael Eisenberg, MD[1], Jillian Henderson, PhD, MPH[2], John Amory, MD, MPH[3], James Smith, MD, MS[4] and Thomas Walsh, MD, MS[4] (Presented By: Michael Eisenberg)
[1]University of California – San Francisco; [2]Department of Obstetrics/Gynecology, University of California – San Francisco; [3]Department of Internal Medicine, University of Washington; [4]Department of Urology, University of California – San Francisco

Introduction and Objectives: Over 500,000 vasectomies are performed annually in the United States. The safety, efficacy, and low complication rate make vasectomy a good family planning option, yet the factors related to use of male surgical sterilization are not well understood. Racial and ethnic differences in vasectomy rates are examined in this analysis to explore whether health care, socioeconomic, or personal and relationship characteristics could account for observed disparities in use.
Methods: We analyzed data from the male sample of the 2002 National Survey of Family Growth to examine the use of vasectomy among the sample of men aged 30-45 (n = 2,161). All analyses accounted for the complex survey design of the NSFG. Demographic, socioeconomic, and reproductive characteristics were analyzed to assess associations with vasectomy. Multivariate logistic regression modeling was used to test the independent associations of significant correlates while adjusting for important confounding and mediating effects of age, marital status, reproductive history, and partner characteristics.
Results: 11.4% of men aged 30-45 years reported having a vasectomy, representing approximately 3.6 million American men. While 14.1% of white men had a vasectomy, only 3.7% of black and 4.5% of Hispanic men reported vasectomy. On multivariate analysis, a significant difference in the odds of vasectomy by race/ethnicity remained, with black (OR 0.20, 0.09-0.45) and Hispanic men (OR 0.41, 0.18-0.95) having a significantly lower rate of vasectomy independent of demographic, partner, and socioeconomic factors. Having ever been married, fathering two or more children, older age, and income >$50,000/year were all associated with vasectomy. Religion, insurance status, and education level were not associated with vasectomy. Having a partner who underwent tubal ligation was not associated with male vasectomy in bivariate analysis. However, in the multivariate analysis, a partner with tubal ligation significantly reduced the odds of vasectomy (OR 0.16, 0.07-0.36).

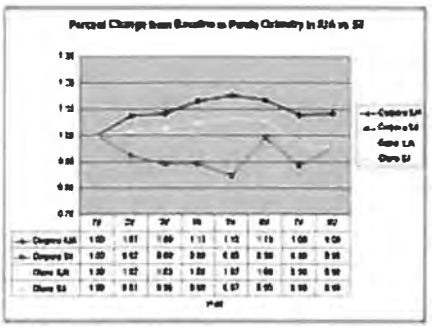
Conclusions: After accounting for reproductive history, partner, and demographic characteristics, black and Hispanic men were less likely to rely on vasectomy for contraception. Further research is needed to identify the reasons for these race/ethnic differences to identify factors that impede and facilitate minority men's reliance on this very safe, effective means of controlling fertility.

9

THE LONGITUDINAL EFFECTS ON PENILE OXYGEN SATURATION: A PROSPECTIVE RANDOMIZED STUDY OF THE NIGHTLY USE OF INTRAURETHRAL ALPROSTADIL VS SILDENAFIL FOLLOWING NERVE SPARING RADICAL PROSTATECTOMY (NSRP)

Joseph Alukal, MD, Brianne Goodwin, BS, Artrit Bytyci, BS and Andrew McCullough, MD (Presented By: Joseph Alukal)
New York University

Introduction: Early penile rehabilitation is recognized as an important part of the recovery of erectile function after RP. Postoperative intracorporal and intraurethral alprostadil as well as oral sildenafil have been reported to improve recovery of erectile function. Enhanced penile oxygenation is believed to be an important factor in the observed beneficial effect. The purpose of this study was to examine the longitudinal effect of nightly sildenafil or intraurethral prostaglandin on flaccid penile oxygen saturation.
Methods: A subgroup of 50 men enrolled in a larger (81 men) randomized comparative 11 month penile rehabilitation trial of nightly alprostadil (MUSE®) 250 mcgm vs sildenafil 50 mgs , underwent penile oximetry preoperatively (V1), at post op visit week one (V2), month 1.25 (V3), month 3 (V4), month 6 (V5), month 9 (V6), month 10 (V7), and month 11 (V8). Medications were started at catheter removal and continued through month 9. After a one month washout, men were challenged with 6 doses of Sildenafil 100 mg. At each visit oximetry was done with an FDA approved tissue oximeter at five sites, the right thigh, (RT), right corpora, (RC), glans penis, (G), left corpora,(LC) and left thigh,(LT).
Results: All men were preoperatively potent (Mean IIEF=29) and underwent nerve sparing prostatectomy by two surgeons (HL) and (ST). Average age was 55. Right and left thigh oximetry did not change significantly over the 11-month period. Corporal oximetry in the IUA cohort increased over 9 months, achieving statistical significance over the second visit nadir throughout the treatment period. Corporal oximetry gradually decreased from baseline in the sildenafil group but returned to baseline by the end of the study.
Conclusion: Despite the short half life of intraurethral alprostadil, low dose nightly alprostadil increased flaccid penile oximetry throughout the study. Flaccid penile oximetry in the sildenafil cohort decreased in the same period. This supports the early incorporation of IUA for penile rehabilitation after RP to maintain corporal oxygenation.



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REAL-TIME PERIPROSTATIC TISSUE IMAGING WITH MULTIPHOTON MICROSCOPY FOR IMPROVING POTENCY OUTCOMES DURING NERVE-SPARING RADICAL PROSTATECTOMY: INITIAL RESULTS FROM FRESH HUMAN PROSTATECTOMY SPECIMENS

Gerald Tan, MB, ChB, MRCSEd, M Med, FAMS[1], Rajiv Yadav, MD[1], Michael Herman, MD[1], Sushmita Mukherjee, PhD[1], Frederick Maxfield, PhD[1], Watt Webb, DSc[2] and Ashutosh Tewari, MD, MCh[1] (Presented By: Gerald Tan)
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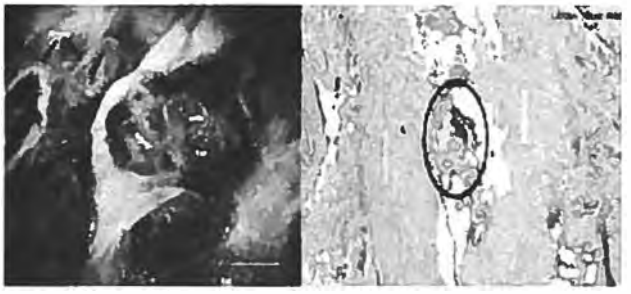
Aim: Multiphoton microscopy (MPM), combined with second harmonic generation (SHG), is a novel technology that permits bioimage acquisition using several low-energy photons to induce autofluorescence of cellular components without use of exogenous stains nor damage to live tissue. With promising results established in our adult male Sprague-Dawley rat model, we now report our initial experience with fresh human prostatectomy specimens.
Patients and Methods: 16 fresh ex vivo human prostatectomy specimens were imaged under an Olympus X61WI upright fluorescence microscope. A femtosecond pulsed Titanium/sapphire laser at 780nm wavelength was used to excite the periprostatic cellular tissue. SHG signals were collected at 390 (+/-35) nm and autofluorescence registered at 380-530nm. Tissues were labeled and correlated with final images obtained at HandE histopathologic confirmation. Institutional review board approval was obtained prior to study commencement.
Results: High-resolution images of the prostatic capsule, periprostatic vessels, smooth muscle cells, and periprostatic inflammation were documented. Histopathologic confirmation of these structures with HandE was closely congruent with images obtained at MPM.
Conclusions: Multiphoton microscopy with SHG delivers superior real-time high-resolution cellular bioimages. Our pilot feasibility study demonstrates the potential for improving potency and cancer clearance outcomes during radical prostatectomy through augmented real-time visualization / preservation of the periprostatic structures with eventual integration of the technology into laparoscopic and robotic platforms.



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EFFECTS OF LIFESTYLE EXPOSURES AND BODY MASS INDEX ON SPERM QUALITY PARAMETERS OF FERTILE MEN

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(Presented By: Karlene Lavelle)
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Spermatogenesis is vulnerable to disruption. Some sperm quality studies have reported unfavorable trends in male reproductive health indicators, and lifestyle exposures (LE) and excess body adiposity have been among the factors implicated. LE (cigarette smoking, alcohol consumption and caffeine intake) have been evaluated in previous epidemiologic sperm studies, typically among men attending infertility clinics, but results of these studies have been inconsistent. Excess body fat, as measured by body mass index (BMI), is associated with many adverse health conditions, however, relatively few studies have examined BMI and sperm quality. Here we explored the potential associations between these four exposures and multiple sperm quality measures among fertile men.

We analyzed standard measures of testicular function (sperm count, concentration, and morphology), as well as Sperm Chromatin Structure Assay measures of DNA integrity (%DFI) and sperm maturity (%HDS), from a community sample of 229 fertile men 18 to 40 years of age. A telephone interview was used to obtain data on LE and potential confounders. We used multiple linear and logistic regression to assess the relationship between LE, BMI and the five sperm outcomes. In this group of fertile men, sperm quality was generally good: sperm count (348 ± 296) million/mL, percent normal morphology (14 ± 6), %DFI (19 ± 12) and %HDS (8 ± 5) (mean ± standard deviation). Results did not reveal significant decrements in sperm outcomes related to smoking status (current, former, never) or BMI, after confounder adjustment. Unexpectedly, former smokers had significantly lower proportions of immature sperm cells compared to non-smokers (-2.8%; 95% Confidence Limits (CL): -4.7%, -0.9%), and obese men (BMI = 30) exhibited, on average, higher sperm concentrations than men of normal weight (BMI 18.5-24.9) (16.2%; 95% CL: 2.0%, 32.3%). Sperm outcomes were generally more favorable among alcohol drinkers than non-drinkers. Conversely, men who reported light (1-150 milligrams/day) or heavy (>300 milligrams/day) daily caffeine intake had, on average, significantly lower proportions of morphologically normal sperm cells relative to non-caffeine drinkers (light: -2.8%; 95% CL: -4.9%, -0.6%; heavy: -2.6%; 95% CL: -4.9%, -0.3%). Results suggest that LE and BMI vary considerably with respect to their independent effects on clinically-relevant markers of testicular function and germ cell DNA integrity among fertile men.

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DEVELOPMENT OF A NOVEL MICROSURGICAL MOUSE MODEL FOR THE STUDY OF VARICOCELE PATHOPHYSIOLOGY

Howard Kim, MD[1], Philip Li, MD[1], Chantal Sottas[2], Renshan Ge, MD[2] and Marc Goldstein, MD[1] (Presented By: Howard Kim)
[1]Weill Cornell Medical College; [2]The Population Council

Introduction and Objectives: The pathophysiology of varicocele is incompletely understood. Our aim was to develop a mouse model for varicocele. Established varicocele models include rat, rabbit, dog and monkey. The advantages of a mouse model include lesser expense and the availability of knockout strains.
Methods: Young adult C57/BL6 mice (8 to 10 weeks) were divided into sham surgery (n=6) and varicocele induction surgery (n=7) groups. After anesthesia, a laparotomy was performed. Under 25x magnification, the left spermatic vein was identified. A 10-0 nylon microsuture needle was used to separate the left spermatic vein from the adherent artery and to ligate the vein near its insertion into the vena cava. Sham animals were treated as above except the left spermatic vein was not ligated. Varicocele induction verification and serum and tissue harvesting were performed at 20 weeks after surgery. Preoperative and postoperative left spermatic vein diameters and testis volumes were compared. Postoperative serum and intratesticular testosterone (T) concentrations were measured with radioimmunoassay.
Results: In the control group, mean vein diameter did not change significantly after surgery (0.38 mm preoperatively vs. 0.28 mm postoperatively, p=0.14). In the test group, mean vein diameter increased from 0.43 mm to 0.58 mm; the difference was not statistically significant (p=0.43). In the control group, mean testis volume did not change significantly after surgery (202 mm3 vs. 197 mm3, p=0.55). In the test group, mean testis volume decreased from 210 mm3 to 112 mm3 (p=0.01). Mean serum T values for the control and test groups were 1.03 ng/ml and 0.81 ng/ml, respectively (p=0.73). Mean intratesticular T values for the control and test groups were 0.14 ng/ml and 0.13 ng/ml, respectively (p=0.78).
Conclusions: Postoperative increase in spermatic vein diameter and decrease in testis volume, serum and intratesticular T levels were selected as markers for successful varicocele induction based on clinical and experimental data in the literature. The postoperative decrease in mean testicular volume was significant. Although not statistically significant, the other parameters all demonstrated a trend toward successful varicocele induction. Additional studies with larger sample sizes are needed to confirm these preliminary findings.
Supported by: The Ferdinand C. Valentine Fellowship for Research in Urology of the New York Academy of Medicine

Sunday, April 5, 2009
11:00 a.m. – 12:30 p.m.

Poster Session I
Location: Grand Ballroom, 2nd Floor

SPERM FUNCTION / SEMEN ANALYSIS

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DETECTION OF SOLUBLE ADENYLYL CYCLASE (ADCY10) HOMOLOG PROTEINS IN BOAR SPERMATOZOA

Kazumi Nakamura, BS[1], Chihiro Suzuki, BS[2], Shunsuke Tate, MS[3], Jibak Lee, PhD[3] and Hiroshi Harayama, PhD[1] (Presented By: Hiroshi Harayama)
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In mammalian spermatozoa, cyclic adenosine 3',5'-monophosphate (cAMP) is an important intracellular messenger that regulates their fertilization-related events, including flagellar movement, capacitation, hyperactivation and acrosomal exocytosis. A sperm soluble adenylyl cyclase (Adcy10) is a unique isoform of enzymatic producers of cAMP which is distinguished from the other isoforms (Adcys1-9) by the G protein-independent activation and lack of membrane-binding domain. Specifically, this isoform is distributed in the sperm cytoplasm and activated by the direct binding with bicarbonate and calcium. Thus, it is generally believed that Adcy10 is a main regulator for the upstream parts of sperm cAMP signaling cascades, although information regarding localization and characteristics of this cyclase has not been reported in the spermatozoa from most animal species except rodent spermatozoa. The aim of this study is to provide evidence for existence of functional Adcy10 homolog proteins in boar spermatozoa. Experiments with RT-PCR techniques, nucleotide sequence analyses and Northern blot analyses have revealed that boar testes exclusively express approximately 5.1-kbp RNA of which nucleotide sequence is highly similar to that of human ADCY10 (sequential homology rate: 85%). Database analyses with the conserved domain architecture retrieval tool (CDART) have suggested that pig Adcy10 homolog protein contains two catalytic domains of adenylyl cyclase in the N-terminal region. Western blot techniques and indirect immunofluorescence with a specific antiserum to recombinant pig Adcy10 homolog proteins have shown that a 48-kDa truncated form of pig Adcy10 homolog protein is localized in the equatorial segment and connecting piece of boar ejaculated spermatozoa. Finally, cell imaging techniques with a cell-permeable calcium indicator (fluo-3/AM) have indicated that incubation with sodium bicarbonate (a physiological activator for Adcy10) can initiate the calcium influx in the boar sperm head that is controlled via the cAMP signaling cascades. These results are consistent with the indication that functional Adcy10 homolog proteins exist in the head of boar spermatozoa.
This work was supported in part by a Grant-in-Aid (20580310) from the Japan Society for the Promotion of Science to HH.

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SMALL UBIQUITIN-RELATED MODIFIER-1 (SUMO-1) IN EJACULATED SPERMATOZOA: A NEW MODULATING FACTOR OF SPERM MOTILITY?

Monica Muratori, Lucia Giuliano, PhD student[1], Sara Marchiani, PhD[1], Lara Tamburrino, PhD student[1], Daniele Nosi, PhD[2], Gianni Forti, MD[1] and Elisabetta Baldi, PhD[1] (Presented By: Monica Muratori)
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SUMO-1 is a member of ubiquitin-related family of proteins and is involved in several physiological functions, including spermatogenesis, where a role in heterochromatin organization and gene expression has been suggested (La Salle et al, 2008). In somatic cells, SUMOylation/deSUMOylation processes are involved in maintenance of a correct mitochondrial function (Zunino et al, 2007). In view of the important role of mitochondria in the maintenance of sperm motility, we investigated the occurrence of SUMOylated proteins in ejaculated sperm and its possible role in sperm functions. By using an anti-SUMO1 antibody we evaluated SUMOylation by flow cytometry, western blot and confocal microscopy analysis in ejaculated human spermatozoa from 31 (12 normo (N)-, 6 terato (T)-, 9 astheno-terato (AT)-, 2 astheno (A)-, and 2 oligo-asthenterato (OAT) zoospermic men undergoing semen analysis for couple infertility in our laboratory. On average, the percentage of SUMOylated sperm was (mean+SD): 10.6+8.1 in N, 13.8+14.5 in T, 22.1+11.1 in AT (P=0.004 vs. N) and 11.39+12.4 in A. Percentage of SUMOylated spermatozoa were significantly inversely correlated with total (r=-0.43, p=0.015) and progressive motility (r=-0.53, p=0.002) and positively with the percentage of immotile spermatozoa (r=0.46, p=0.008), but not with sperm concentration or other parameters of semen analysis. Interestingly, the percentage of SUMOylated spermatozoa was also negatively correlated with age of patients (r=-0.41, p=0.02). Western analysis demonstrated the presence of several SUMOylated proteins in a molecular weight range of 25-80 kDa. By immunoconfocal analysis we found that SUMOylation is present in the post-acrosomal region and, above all, in the midpiece of human spermatozoa, consistent with a possible localization in mitochondria. Recently, it has been demonstrated that persistent SUMOylation of the mitochondrial protein DRP1, by silencing the de-SUMOylating enzyme SENP5 in somatic cells, heavily affects mitochondrial functions (Zunino et al, 2007). In this context, our data suggest that SUMOylation represents a new parameter involved in the regulation of sperm motility.

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ARE ALL CREATED EQUAL? IS IT POSSIBLE TO "SUPER-SELECT" THE MOST VIABLE ONES FROM A SAMPLE OF SPERM WITH MINIMAL MOTILITY?

Maurice Garcia, MD[1], Aaron Ohta, MD[2], Thomas Walsh, MD[1], James Smith, MD[4], Ming Wu, MD[2] and Tom Lue, MD[3] (Presented By: Maurice Garcia)
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Background and Purpose: We have previously shown that non-motile sperm can be sorted to separate non-viable sperm from viable sperm, using a novel non-invasive DEP-based sperm-sorting platform. Because our sorting platform appears to stratify the sperm population into "viable" and "non-viable" sperm groups, and thus appears, at this point, to sort based on "viability", we concluded that if viable sperm respond variably to our DEP-based sorting method, then it is possible that the our sorting method could be used to identify which sperm, among the sorted "viable" sperm, are "more viable", and which are "less viable".

Materials and Methods: Fresh ejaculate specimens from 6 healthy men were assessed. Specimens were incubated with 0.4% Trypan Blue. Using a custom-designed sorting chip, 55 consecutive randomly selected sperm were assayed from each specimen: Motile sperm (N=5); Non-motile Trypan Blue negative (N=25) and positive (N=25) sperm. We recorded sperm response to DEP. We also measured the magnitude of each non-motile sperm's response (attraction or repulsion) to the DEP energy field. We also determined the maximal velocity (MV) (im/s) at which the non-motile sperm could be moved across the field by attraction or repulsion to DEP.
Results: A total of 330 individual sperm were individually assayed. We confirmed previous results showing that live sperm could be sorted from dead sperm. Among non-motile sperm, measured response to DEP was variable: Trypan-positive sperm demonstrated either a neutral (57/125=46%) or a weakly repulsive (68/125=54%) response to DEP; mean MV= -1.04 im/s (SD 0.93). All sperm that responded with attraction to DEP were Trypan-negative (125/125). Mean MV = +8.07 im/s (SD 3.83), ($p<0.00001$). A fraction of Trypan-negative non-motile sperm (15%) demonstrated no response to the DEP.
Conclusions: Our modified DEP cell-sorting platform is capable of non-invasively assessing, sorting, and retrieving viable from non-viable sperm for ICSI. The significant SD of the attraction-response of viable non-motile sperm to DEP suggests that it may be possible to sub-stratify viable sperm based on "relative viability". If future studies confirm that a greater magnitude of attraction to DEP correlates with greater viability (less DNA damage and/or improved fertilization outcomes), then it may be possible to "super-select" the most viable sperm from a sample with limited overall sperm motility, for use with ICSI.

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EFFECT OF MUCUNA PRUREINS ON INTRACELLULAR REACTIVE OXYGEN SPECIES, STRUCTURAL AND FUNCTIONAL INTEGRITY OF EPIDIDYMAL SPERMATOZOA IN AGED RAT

Seppan Prakash, MSc, PhD, Sekar Suresh, MSc and Elumalai Prithiviraj, MSc (Presented By: Seppan Prakash)
University of Madras

Mucuna pruriens Linn. (M. pruriens), a leguminous plant, has been recognized as an aphrodisiac and spermatogenic agent. Protective efficacy of M. pruriens against ROS induced pathophysiological alterations in aged rat epididymal sperm. Wister albino rat was analyzed. Animals were grouped as group I, II, III and IV i.e. young, aged, aged treated with ethanolic extract (200 mg/kg b.w.) of M. pruriens and young rats treated with M. pruriens respectively. At the end of the experimental period i.e. 60 days, animals were sacrificed; epididymal sperm were collected and subjected to count, viability, motility, morphology and morphometric analysis. Enzymatic and non-enzymatic antioxidants, reactive oxygen species (ROS), lipid peroxidation (LPO), DNA damage, chromosomal integrity and mitochondrial membrane potential were estimated. Results obtained from the aged animal showed significant reduction in sperm count, viability and motility, increased morphological damage and increase in the number of sperm with cytoplasmic remnant and these alterations were significantly reversed in M. pruriens treated group. Significant increase in LPO, HO., and H2O2 production and significant decline in the levels of the enzymatic and non-enzymatic antioxidants were observed in the aged animals. Supplementation of M. pruriens significantly reduced ROS and LPO production and significant increase in the both enzymatic and non-enzymatic antioxidant levels. No toxic manifestation was observed in group IV. There were significant DNA damage, loss of chromosomal integrity and increase in mitochondrial membrane permeability in aged rat sperm. This was significantly reduced in group III. Present observation indicates the antioxidant enhancing property, free radical quenching ability and spermatogenic efficacy of the M. pruriens. Collectively sperm damage in ageing was significantly reduced by quenching ROS, improving antioxidant defense system and mitochondrial function. This signifies the therapeutic role of M. pruriens on sperm in ageing and andropause subjects.

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NA+K+-ATPASE AND CAPACITATION SIGNALLING IN BOAR SPERMATOZOA

Meghan Mackenzie Bell, BSc and Mary Buhr, PhD (Presented By: Meghan Mackenzie Bell)
University of Guelph

Introduction and Objective: Spermatozoa must complete capacitation to be able to fertilize an oocyte. Our lab has shown that the transmembrane protein Na+K+-ATPase, in addition to its traditional enzyme function, acts as a signaling molecule, and the steroid hormone ouabain, a highly specific ligand for Na+K+-ATPase, induces capacitation in bull sperm. Na+K+-ATPase contains alpha and beta subunits, whose isoforms have been localized in bovine spermatozoa. The current objective was to explore Na+K+-ATPase's role in porcine capacitation.
Methods A minimum of 1 fresh ejaculate from each of 4 mature boars of proven fertility was used per experiment. Ouabain's interaction with Na+K+-ATPase subunits was assessed by incubating sperm with 0 - 500µM ouabain. Binding of alpha isoforms 1, 2, 3 and beta 1, 2, 3 were determined by immunofluorescence after 10 minutes. Ouabain's effects on capacitation were assessed microscopically on sperm incubated for 0, 4 or 6 hours in capacitating conditions with 0, 0.01, 50 or 100µM ouabain.
Results: Ouabain lowered the visual fluorescence intensity of alpha subunits 1 and 3, and did not affect the detected presence of the isoforms alpha 2 or beta 1-3. As expected, significantly more sperm underwent an ionophore-induced acrosome reaction (AR) after 6 hours of incubation in capacitation medium versus control (BTS). Ouabain increased the spontaneous (non-ionophore induced) AR in spermatozoa incubated in BTS, but not capacitating media, in a time dependent manner (0hr incubation, 15 vs.33 ± 1.7%AR, mean ± SE, 0 vs. 100 uM ouabain, respectively; P=0.01), compared to 6hr (26 vs. 40 ±1.67 %AR, 0 and 100 uM ouabain, P<0.0001). In capacitation media, although at time 0 the % AR at 0 uM ouabain was identical to the BTS treatments, ouabain caused a significant drop in %AR at 6 hours to 8 ± 1.7% AR (P<0.0001).
Conclusions: Na+K+-ATPase is operative in boar sperm capacitation, with its specific ligand ouabain interacting preferentially with the alpha 1 and/or 3 isoforms to influence the onset of capacitation in porcine spermatozoa.
Funding provided by NSERC, OMAFRA and YSTOP

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REACTIVE OXYGEN SPECIES AND BOAR SPERM FUNCTION

Basim Awda, PhD, Meghan Bell, BSc and Mary Buhr, PhD (Presented By: Basim Awda)
University of Guelph

Introduction and Objectives: Boar spermatozoa are very susceptible to Reactive Oxygen Species (ROS) due to their high content of polyunsaturated fatty acids. This study hypothesized that cryopreservation of boar spermatozoa, and exposing fresh boar spermatozoa to Xanthine/Xanthine Oxidase (XA/XO) ROS generating system, generate ROS, induce membrane lipid peroxidation and phospholipase A (PLA) activity, and impair such whole sperm functions as motility, viability, and onset of the acrosome reaction.
Methods: Multiple ejaculates from different boars were used to measure intracellular levels of superoxide anion (O2Ë™Ë‰) and hydrogen peroxide (H2O2) by flow cytometry using hydroethidine (HE) and 2',7'-dichlorodihydrofluorscein diacetate (H2DCFDA) respectively; sperm lipid peroxidation and PLA activity were determined by flow cytometer by labeling sperm with BODIPY 581/591 C11 and bis-BODIPY FL C11 respectively, counterstaining for viability. Motility was measured by computer assisted analysis, and acrosome reaction by Coomassi staining after 0 and 4 hours in BTS buffer or capacitating media.
Results: Exposing boar sperm to the XA/XO ROS generating system rapidly increased H2O2 and lipid peroxidation in all sperm, increased PLA levels in dead sperm, and had no effect on sperm intracellular production of O2Ë™Ë‰. Sperm simultaneously became immotile, but viability remained high and many more sperm underwent the acrosome reaction in capacitating incubations. Cryopreservation decreased sperm motility, viability and intracellular levels of O2Ë™Ë‰ but had no effect on H2O2.
Conclusions: Hydrogen peroxide is the major free radical mediating the response of boar spermatozoa to the XA/XO ROS generating system, but not cryopreservation. Boar sperm motility, acrosome integrity and lipid peroxidation are more sensitive indicators of oxidative stress than viability and PLA activity. ROS may stimulate the acrosome reaction in boar sperm through membrane lipid peroxidation and PLA activation.
The authors gratefully acknowledge funding from NSERC and OMAFRA.

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GENOME-WIDE APPROACHES TO IDENTIFYING SPERM BIOMARKERS OF TOXICANT EXPOSURE

Sara Pacheco, BS and Kim Boekelheide, MD, PHD (Presented By: Sara Pacheco)
Brown University

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 is to use two animal models of infertility to develop sperm biomarkers of effect for exposures to specific Sertoli and germ cell toxicants. Methods: Groups of rats (n=10) were chronically dosed for 12 weeks with either 0.33% 2,5-hexanedione (HD) or 5 mg/kg/d 1,2-dibromo-3-chloropropane (DBCP). Comparing sperm samples extracted from control and toxicant treated rats using traditional sperm analysis, pyrosequencing, gene array analysis, and RT-PCR will allow us to test the following working hypothesis: The mRNA and epigenetic profiles of sperm DNA will be unique for each cell type specific testicular toxicant and will provide increased sensitivity and diagnostic specificity compared to current markers of fertility status.
Results: Exposure to HD caused significant decreases in overall body weight, testis weights, and epididymal weights compared to control. Exposure to DBCP also caused significant decreases in overall body weight, testis weights, and epididymal weights compared to the corn oil control. Even though there was a significant difference in overall body weight, there were no discrepancies among the testis and epididymal weights of the two control groups. In our preliminary experiments, quantitative RT-PCR identified increased expression of bcl-2 and protamine-1 mRNA in sperm from rats exposed to HD for 9 weeks, compared to control. The microarray and epigenetic experiments are currently in progress. Conclusions: These experiments will help to develop the background knowledge needed to interpret molecular and epigenetic signatures of human sperm samples from control (pre-vasectomized) and infertile men.

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UBIQUITOUS HYALURONIDASESARE PRESENT IN HUMAN AND MOUSE SPERM WHERE THEY ARE ENZYMATICALLY ACTIVE

Kristen Reese, BS, Rolands Aravindan, PhD, Griffiths Genevieve, PhD, Shao Minghai, PhD, Bruton Jessica, BS, Woody Sarah, BS, Barran Diniece, BS, Naik Tejal, BS, Galileo Deni, PhD and Patricia Martin-DeLeon, PhD (Presented By: Patricia Martin-DeLeon)
Department of Biological Sciences, University of Delaware

Mammalian hyaluronidases (hyases) are a family of six enzymes that catalyze the breakdown of hyaluronan or hyaluronic acid (HA) which is abundantly present in the extracellular matrix. Two unlinked gene clusters encode these multifunctional proteins, three of which comprise the somatic (or ubiquitous) acid-active subset and three the neutral-active reproductive group that is present in sperm. Of the six hyases, five are expressed in the testis. However, currently only the three neutral-active

hyases, exemplified by Sperm adhesion molecule 1 (SPAM1 or PH-20), are considered to be sperm hyases and in humans. Unlike mice, SPAM1 is the only identified sperm hyase. Here we show that Hyaluronidase 2, and 3 (HYAL2 and HYAL3) are present in human and mouse sperm, as detected by indirect immunofluorescence and flow cytometry. Western analysis showed that HYAL3 exists in two isoforms in mature human (~47 kDa, ~55 kDa) and mouse (~44, ~47kDa) sperm where it resides on the head and midpiece of the flagellum. It is present in both the soluble and insoluble membrane fractions after the acrosome reaction (AR), with the isoforms differentially distributed, and is most abundant in the AR fraction. Zymography of Hyal3 null and wild-type (WT) sperm proteins shows that HYAL3 contributes to hyase activity at pH 3-7 for the major protein fractions. At pH 3-4 Hyal3 null sperm showed markedly decreased activity compared to WT and in humans hyase activity was higher at acidic than at neutral pH. HYAL3 deficiency leads to a delay in cumulus penetration and a reduction in acrosomal exocytosis in vitro, although Hyal3 nulls are fully fertile with natural mating. HYAL3 is expressed in uterine and epididymal fluids from which it is acquired in vitro by caudal mouse sperm. Western shows a 54 kDa mouse HYAL2 that is distributed in both the soluble and insoluble fractions after the AR; and for Hyal3 null sperm proteins a similar band is seen with hyase activity at pH 4. This suggests that HYAL2 may be uipregulated in Hyal3 nulls. Based on expression and functional activities, a re-definition of reproductive hyases is in order. Our findings underscore the polygenic nature of steps essential in the fertilization process and may be relevant t o the treatment of sperm pathology in IVF.

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ADJUDIN IS A PROSPECTIVE CANDIDATE OF SPERMICIDE

Hui Wang, PhD Candidate, Xiangxiang Chen, Master, Zuomin Zhou, PhD, Xuejiang Guo, PhD, Fuqiang Wang, Master, Jing Xie, Master and Jiahao Sha, PhD (Presented By: Hui Wang)
Correspondence

Recently, several investigators have focused on inhibition of sperm motility as a promising target for contraceptive development. This nonhormonal approach to contraception might be very specific and has a very rapid onset of action with the lack of systemic side effects. Adjudin, formerly known as AF-2364, (1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide), is a potential male contraceptive when administered orally to adult Sprague–Dawley rats. This compound induces reversible germ cell loss from the seminiferous epithelium by disrupting cell adhesion function between Sertoli and germ cells. Using a FSH mutant as a carrier, AF-2364 can be delivered specifically to the testis which makes Adjudin become a male contraceptive for human use. In the present study, we explored the possibility of AF-2364 as a spermicide and its primary mechanism in spermicidal process. AF-2364 blocked the human sperm motility in-vitro (from 8ig-32ig/50×106 sperm) and this regulation was concentration and time dependent. 2-D electrophoresis and Mass Spectrometry was employed to study the differentially expressed human sperm proteins after the treatment of AF-2364. Further, the ATP quantity released from human sperm were significantly different ($P<0.05$) between the AF-2364 treating group and the control group. This might be one of the possible reasons for AF-2364 induced impaired sperm motility. Besides, we also observed the similar concentration and time dependent sperm motility blocking phenomenon of AF-2364 (8ig/5×106 sperm) on BALB/C mice which makes the clinical research of AF-2364 as a spermicide to be possible. In conclusion, AF-2364 has a spermicidal function both to human being and mice and it might be developed to be a new contraceptive gel.

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VAMP4, A SPERM MOTILITY RELATED V-SNARE PROTEIN

Jun Xing, PhD candidate[1], Jian Shen, PhD candidate[2], Xuejiang Guo, PhD candidate[2], Zuoming Zhou, PhD[2] and Jiahao Sha, PhD[2] (Presented By: Jun Xing)
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In somatic cell, most energy consumes comes from glucose, which is mainly taken through the v-snare related glucose transporter (GLUT)-mediated endocytosis; and GLUT family is known to play an important role in the process. Motility of sperm need large amount of energy, and glucose is one of the most important resources for energy. Although some researches on the localizations of the GLUT family proteins in sperm have been reported, it is still not known whether the glucose uptake involves the GLUT-mediated endocytosis mechanism similar to that in somatic cells. In our research, we found that VAMP4, a v-snare protein, was located not only in acrosome but also in the midpiece of mouse sperm. The antibody inhibition assay indicated that the motility was significantly decreased in sperm incubated with VAMP4 antibody compared with that in sperm treated with IgG or irrelevant antibody. Additionally, VAMP4 antibody doesn't influence the sperm survival as analyzed by eosin stain. The penetration assay of mouse sperm through zona-free mouse eggs was performed. And the group with un-capacitated sperm cultured with VAMP4 antibody showed significantly decreased rates of fertilization compared with control IgG antibody. sperm gets its motile ability in epididymis. Immunofluorescence of sperm from caput epididymidis and cauda epididymidis showed that the signal of VAMP4 increased as the sperm proceeded from caput epididymidis to cauda epididymidis, which implied the possible role VAMP4 in acquisition of sperm motility. In the transmission electron microscopic study, we have found a lot of small vesicles in the sperm midpiece, the section consumes massive energy, which was not reported before. Thus, it is possible that the sperm tail probably take glucose through the GLUT-mediated endocytosis mechanism similar to that in somatic cells. Antibody inhibition assay showed that the glucose uptake decreased in sperm treated with the VAMP4 antibody, which indicated glucose might be taken through the v-snare related receptor-mediated endocytosis in sperm. Our study showed the possibility for the first time that the glucose uptake, which was necessary for the sperm motility, might be though the VAMP4-related GLUT-mediated endocytosis.

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CAN SPERM CHROMATIN PACKAGING INFLUENCE RESULTS OF SPERM DNA INTEGRITY MEASURES? RESULTS OF THE TUNEL ASSAY

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Introduction and Objectives: The DNA in the sperm is protected by the condensed status of the chromatin. Protamines and Zn2+ are important for this sperm specific chromatin stability. The condensed structure is essential since the spermatozoon does not have an active system for repair of DNA strand breaks occurring during the transfer from the testicle to the egg. The more strand breaks that must be repaired simultaneously in the ooplasm the greater the risk for faulty repairs, which can affect both the child to be and grandchildren. TdT-mediated dUTP Nick End Labeling (TUNEL) was originally developed to detect apoptosis in somatic cells, but has in recent year been used as a marker for spermatozoa with presumed DNA strand-breaks. The purpose of this investigation was to evaluate if TUNEL results could be influenced by other factors than pre-existing sperm DNA strand breaks.
Methods: Spermatozoa from ten semen samples were obtained from patient samples (infertility investigations) where consent had been given to research purposes after diagnostic procedures had been finished.

Spermatozoa in seminal plasma were fixed, permeabilized and incubated with deoxynucleotidyl tranferase and nucleotides incorporated with fluoresceine- as well as DNA-binding propidium iodide. Images were captured (Slidebook Application 4.1.0) using fluorescence microscopy with transmission filter for fluoresceine (green) and propidium iodide (red), and contrast enhancing filter to identify the sperm. Images were analyzed using Picsara 8.9 and spermatozoa were classified as positive or negative. Statistical analyses were performed using GraphPad Prism 5.01.
Results: Only 15-53 % of the sperm in DNA-ase treated positive controls were able to respond to the TUNEL method. The proportion of TUNEL positive sperm in the samples increased with longer time of abstinence and with longer time between ejaculation and analysis. A negative correlation between semen zinc concentration and frequency TUNEL positive spermatozoa
Conclusions: The TUNEL assay only measures a subpopulation of the spermatozoa in a given semen sample. Insufficient availability of DNA has not earlier been considered when TUNEL has been used to determine the frequency of sperm DNA damage and calls for a positive control for every analyzed sample. Zn2+ in semen can either protect the sperm DNA or reduce the access to the sperm DNA of the TUNEL assay, since TUNEL positivity decreases with higher seminal Zn2+.

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THE IMPACT OF CIGARETTE SMOKING ON SPERM DNA AND OXIDATIVE STRESS IN NON-LEUKOCYTOSPERMIC INFERTILE MEN

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Introduction: Oxidants in cigarette smoke are thought to damage sperm DNA, and smokers have more oxidative DNA damage in their sperm than do nonsmokers. Because oxidative sperm DNA damage can be induced either by cigarette smoking oxidants or by leukocytes, non-leukocytospermic infertile men smoker may reveal the effects of smoking on sperm DNA integrity, oxidants and antioxidants levels.
Objective: To evaluate the effects of cigarette smoking on sperm DNA integrity, oxidants and antioxidants levels in non-leukocytospermic infertile men.
Methods: Semen samples from non-leukocytospermic infertile men (n = 79; subdivided into infertile smokers (n=43) and infertile non-smokers (n=36) subgroups. A fertile men group (n = 18) were included as a control. All semen samples were subjected to standard semen analysis according to WHO guidelines, sperm DNA analysis by flow cytometry, oxidants (malondialdehyde (MDA) concentration), and antioxidant (superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH)) analysis.
Results: Sperm DNA-fragmentation index (DFI) was significantly higher in the infertile smokers group than in non-smokers infertile and fertile groups (P= 0.032; P=0.001 respectively). Cigarette smoking was significantly correlated with increased MDA, DFI and decreased SOD levels (r=0.796, P=0.0001; r=0.371, P=0.033; r= -0.545, P=0.013 respectively).
Conclusions: Cigarette smoking in non-leukocytospermic infertile men is associated with increased susceptibility of DNA to denaturation causing increased sperm DNA fragmentation that may be sufficient to cause subfertility in the male. These effects may be attributed to increased oxidative stress and insufficient scavenging antioxidant enzymes in the seminal fluid of non-leukocytospermic infertile patients.

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PROSPECTIVE STUDY OF FERTILAID VITAMIN IN MEN WITH LOW SPERM QUALITY

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Introduction and Objectives: A 90-day, randomized, double-blind, placebo controlled study determined if treatment with the vitamin FertilAid for Men (Fairhaven Health, Bellingham, WA) improved sperm quality in men.
Methods: Adult males were enrolled with abnormal sperm parameters defined as one or more: low counts, low percentage of motility; or low percentage of normal morphology. Eligible subjects (including no vitamin ingestion within 30 days) provided two baseline (initial) semen samples. Routine semen analysis was performed according to current WHO guidelines (World Health Organization, 1999) to determine: sperm count per ml and per total ejaculate; percent motile sperm and speed of progression for the motile sperm; and strict assessment of sperm morphology. From these parameters the total motile sperm count and total normal-motile sperm counts for each ejaculate were determined. Following analysis of the baseline semen samples, subjects were randomly assigned to FertilAid or placebo. Following the 90 days of vitamin or placebo therapy, subjects again provided two semen samples. The same laboratory performed the initial and final analyses for each man. Statistical analysis was performed using the Mann Whitney U test.
Results Obtained: No differences existed for sperm parameters between the groups at baseline. Results. A total of 14 subjects completed the trial (initially 10 per treatment were recruited). Eight were randomized to FertilAid and 6 were randomized to placebo. Total normal-motile sperm numbers in the ejaculate improved for men using FertilAid (p=0.05), versus men on placebo. Additionally, the total motile sperm count also showed a tendency towards improvement (p=0.09). Other parameters did not differ between the two groups. Conclusions: In spite of the small sample size in this study, significant improvements were found for men taking FertilAid, with regards to the total number of normal-motile sperm in the ejaculate. Larger studies should be done to confirm the results seen here. Use of FertilAid by the male partner may improve the ejaculate quality in some men, specifically with regards to the number of normally shaped, motile sperm produced. Funded in part by Fairhaven Health.

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DEATH OF HUMAN SPERMATOZOA MAY BE REGULATED SEPARATELY BY MITOCHONDRIAL AND CYTOSOLIC PATHWAYS

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Introduction and Objective: As a terminal cell, cell death is a normal function of spermatozoa. Calcium ionophore A23187 allows divalent cations to cross the cell membrane which leads to the acrosome reaction. A23187 also inhibits mitochondrial ATPase and uncouples oxidative phosphorylation. It is reasonable that A23187 may also induce apoptosis. Staurosporine has been used to induce apoptosis in both somatic cells and spermatozoa. Staurosporine induces apoptosis by non-selective inhibition of protein kinases. The purpose of this preliminary study was to determine if the effects of A23187 and Staurosporine can be differentiated using the common semen parameters of motility, viability, linearity, velocity and acrosome reaction.
Materials and Methods: Aliquots of sperm from normal donors were incubated either in media alone (control), DMSO, staurosporine (10iM final concentration), or A23187 (10IM final) for five hours. At designated time intervals samples were taken and motility, velocity, and linearity were determined using a computer assisted semen analyzer (CASA). Viability was determined using eosin live/dead stain. The state of the acrosome was determined using peanut agglutinin conjugated with FITC.

Results: There was no significant difference between the control and DMSO on any of the parameters. A significant increase in acrosome reaction and a decrease in motility were seen in the sample incubated with A23187 and Staurosporine. Staurosporine had an immediate dramatic decrease in motility and a moderate effect on acrosome reaction. In contrast, A23187 had a moderate decrease in motility but an immediate increase in the number of spermatozoa that were acrosome reacted. Conclusion: Spermatozoa undergo membrane remodeling, acrosome reaction and cell death as part of their normal functions. From this preliminary data it is possible to predict that spermatozoa undergo programmed cell death via pathways that originate within either the mitochondria or in the cytosol/acrosome region of the spermatozoa. Further studies are planned to determine if A23187 and staurosporine elicit differing responses in and localization of apoptotic markers such as: activation of caspases, mitochondrial membrane potential, phosphatidyl serine exposure and DNA fragmentation.

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CAN SPERM CHROMATIN PACKAGING INFLUENCE RESULTS OF SPERM DNA INTEGRITY MEASURES? RESULTS OF THE COMET ASSAY

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Introduction and Objectives: Protamines and Zn2+ are important for the condensed sperm chromatin. Sperm DNA appears to be protected by a sperm specific chromatin stability. Absence of active DNA repair systems increases the risk for faulty DNA repair in the ooplasm that can appear as chromosome abnormalities in later generations.
The purpose of this investigation was to evaluate if COMET assay results can be influenced by other factors than pre-existing DNA strand breaks.
Methods: The Comet assay is supposed to detect strand breaks in sperm DNA. From spermatozoa imbedded in agarose gel DNA is set free by protein extraction with a lysis buffer with high content of salt, detergent, zinc binding EDTA, zinc binding and disulfide breaking DTT, and proteinase K. DNA is moved out of the nucleus by electrophoresis. Images were captured under fluorescence microscopy and analyzed. Size and brightness of "comet" tail indicates the size of the DNA pieces extracted from the head.
In total 34 semen samples were used, obtained from patient samples where consent had been given for research purposes after diagnostic procedures had been finished. In seven samples the content of zinc and structural stability was experimentally varied by exposure to zinc binding and disulfide cleaving cysteine.
Results: Large variety of comet sizes: from no tail to huge tails dissociated from the nucleus core. The variation between different semen samples was considerable. There was no correlation between seminal Zn2+ and the COMET parameters. The proportion sperm heads showing a large and bright comet tail increased among cysteine pre-exposed sperm cf. controls (P=0.01). When cysteine was removed by washing the cells, the proportion sperm with large and bright tail did not differ from controls. The increment in comet tail among cysteine exposed sperm correlated to Zn2+ in seminal plasma.
Conclusions: The reversible cysteine effect on COMET assay results can be due to changes in sperm DNA accessibility; first through destabilization (disulfide bridge breakage and Zn2+ chelation). Reversibility of the access could be due to excessive disulfide bridge formation in the absence of cysteine and zinc. Relation between seminal zinc and increase in comet response during cysteine treatment may be due to decreased disulfide formation and thereby increasing access to the DNA. Thus the COMET assay is influenced by the prevailing type of sperm chromatin stabilization.

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OBJECTIVE COMPARISON OF THE SPERM CLASS ANALYZER® TO MANUAL ASSESSMENT FOR GRADING HUMAN SPERM MORPHOLOGY BY STRICT CRITERIA

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Introduction: Strict morphology is considered a useful tool in predicting intrauterine insemination (IUI) outcome and IVF fertilization rates. However, the standard manual methods for grading sperm result in large intra- and inter- technologist variations, which can diminish the accuracy and therefore the usefulness of this test. Computer-aided sperm analysis (CASA) has been proposed as a method to limit variation. Sperm Class Analyzer ® (SCA) is a CASA that has been used for sperm morphology studies in domestic animals, but for which limited data is available with human sperm. The objective of this study is to compare the SCA to manual assessment of human sperm strict morphology.
Materials and Methods: Donor sperm samples were used to make multiple morphology slides for our QC program. Three of these donors were selected to represent a range of morphologies. Five slides of each donor were then randomly selected and analyzed in triplicate to determine intra- and inter-slide coefficients of variation (CV). All slides were coded. CVs were calculated using the percent abnormal sperm obtained.
Results: The average values from weekly QC measurements were 5.6 +/- 3.1, 10.0 +/- 4.2 and 8.1 +/- 4.2 percent normal for donors C, D and E, respectively. There were no differences between manual and SCA results for sample C and D. However, the SCA returned a result over twice the value obtained manually for sample **E**. Reanalysis of sample **E** identified several features that accounted for the difference, including acrosomal membrane vacuoles and tail defects not identified by the SCA, as well as shape variations called abnormal by manual assessment. Intra-slide variation did not differ between methods; however, inter-slide variation was different for each sample with 2 of 3 samples having higher CVs with SCA analysis. Conclusions: The SCA provides results that are comparable to manual strict analysis, though special considerations must be made regarding vacuoles and tail defects. Assessment of shape may be more consistent with the SCA and may provide a means to become less stringent.

Donor	Mean		Intra-Slide CV		Inter-Slide CV	
	SCA	Manual	SCA	Manual	SCA	Manual
C	4.3 ± 5.7	6.8 ± 3.0	15 ± 0.4	22 ± 0.4	13 ± 0.4	37 ± 0.4*
D	16.0 ± 4.8	17.2 ± 0.7	5.4 ± 1.0	2.8 ± 1.0	7.4 ± 1.1*	17 ± 11
E	20.5 ± 4.7*	8.7 ± 2.8	5.5 ± 0.8	3.4 ± 0.8	6.9 ± 0.8*	4.1 ± 0.8
	*P<.05				*P<05	

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TYROSINE PHOSPHORYLATION OF ACIDIC PROTEINS LOCALIZED TO THE ACROSOME IS ASSOCIATED WITH COLD SHOCK-AND CALCIUM IONOPHORE-INDUCED ACROSOME REACTIONS IN PORCINE SPERM

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University of Pennsylvania School of Veterinary Medicine

Tyrosine phosphorylation of a protein or proteins that migrate to a relative molecular mass (Mr) of 32 x 10^3 has been associated with porcine sperm capacitation. A basic protein, Proacrosin binding protein (sp32), has been identified as a substrate of capacitation-associated tyrosine phosphorylation in porcine sperm. Our previous study demonstrated an increase in porcine sperm protein tyrosine phosphorylation (PY) of protein(s) migrating to Mr 28 x 10^3 on SDS-PAGE following cold shock. The objective of this study was to determine if the same porcine sperm protein undergoes tyrosine phosphorylation following cold shock and incubation under conditions that support capacitation. A total of 3 sets of

experiments using individual semen samples from 3 adult boars were performed. Sperm were suspended in capacitation medium containing 0.04% BSA and 0.8 mM 2-hydroxypropyl-beta-cyclodextrin and either incubated at 39°C for 3 h or equilibrated for 15 min at 30°C, and then transferred to a water bath at 4°C for 10 min (cold shock). Cold shock at this temperature causes AR of nearly all porcine sperm in the sample. Before incubation (0h) and following cold shock (CS), 3h incubation at 39°C (3h) or 3h incubation at 39°C followed by treatment with 2 microM calcium ionophore A23187 to induce the acrosome reaction (AR), proteins were extracted with Laemmli SDS sample buffer and processed for one- and two-dimensional (2D) SDS-PAGE followed by immunoblotting with anti-PY antibody (clone 4G10). Samples were also fixed with 4% paraformaldehyde and processed for indirect immunofluorescence with the same antibody. We report here that anti-PY immunoblots demonstrated an increase in PY of a Mr 28 x 10^3 protein band after cold shock or induced AR, but only minimal increase in PY following capacitation without induced AR. Indirect immunofluorescence localizes anti-PY immunoreactivity to the acrosome in AR, but not intact sperm, after cold shock or capacitation. 2D SDS-PAGE also identifies increased PY only in CS and AR sperm in a series of spots in the acidic range (3.5 to 6.5 isoelectric points) at the same relative molecular mass. We currently await identification of these protein spots by mass spectrometry. This study suggests that the same acidic proteins undergo PY in association with the AR due to cold shock or calcium ionophore treatment of capacitated porcine sperm.
Supported by a grant from PA Dept. Ag. (ME 446735)

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EJACULATE TRAITS AND SEMINAL PLASMA CHEMISTRY OF GOOD VERSUS POOR QUALITY EJACULATES IN ASIAN ELEPHANTS (ELEPHAS MAXIMUS)

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The North American Asian elephant population is not self-sustaining due to poor reproduction. Genetic management of this species is being accomplished to a large extent via artificial insemination using fresh semen. A major challenge continually faced by managers, however, is the high variability in ejaculate quality both across and within bulls. The objectives of this study were to 1) characterize the seminal traits of good (= 55% motility) versus poor (< 50% motility) quality ejaculates and 2) determine if differences in seminal plasma chemistry contribute to variation in ejaculate quality. Ejaculates (n = 87; 12 bulls) were collected by rectal massage and immediately assessed for volume, sperm concentration/ml, percent motility, progressive motility, normal sperm, acrosomal integrity, pH and osmolality. For analysis of seminal plasma chemistry, an aliquot (n = 23 ejaculates; 7 bulls; 5 ml/ejaculate) of raw semen was centrifuged (200 x g; 5 min), and the supernatant aspirated, re-centrifuged (500 x g; 20 min) and stored at -70°C until analysis. Samples were submitted to a commercial diagnostics laboratory for determination of total protein, albumin, SGOT, SGPT, alkaline phosphatase, calcium, chloride, glucose, phosphorus, potassium, sodium, magnesium, and urea nitrogen. All semen parameters except pH (7.1 ± 0.1 vs. 6.95 ± 0.1; Mean ± SEM) and osmolality (271.5 ± 1.7 vs. 268 ± 9.6) were different between good and poor quality ejaculates, respectively. Good ejaculates contained higher ($P < 0.05$) total motility (78.6 ± 1.7% vs. 11 ± 2.3%), progressive motility (68.8 ± 3.9% vs. 9.1 ± 2.2%), spermatozoa with normal morphology (76.9 ± 2.0% vs. 50.0 ± 3.7%), and normal acrosomes (37.7 ± 4.2% vs. 11.0 ± 2.3%) compared with poor ejaculates. Interestingly, good ejaculates contained lower ($P < 0.05$; 589.0 ± 101.9 x 106) sperm concentration per ml compared with poor quality counterparts (1374 ± 92.2 x 106). Analyses of seminal plasma revealed no differences ($P > 0.05$) between good and poor quality ejaculates in seminal plasma chemistry. Results demonstrate that differences in ejaculate quality between good and poor quality ejaculates are not solely determined by seminal plasma chemistry in Asian elephants. These results emphasize the need for further investigations into improving semen collection methods in elephants. (Funding provided by International Elephant Foundation, Feld Entertainment Inc., Indianapolis Zoo and Friends of the National Zoo).

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COMPARISON OF SPERM DNA INTEGRITY BEFORE AND AFTER DENSITY GRADIENT CENTRIFUGATION EMPLOYING A NOVEL TOLUIDINE BLUE ASSAY

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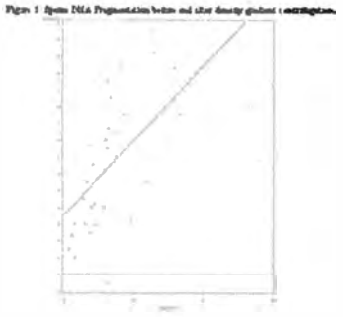
NSLIJ

Objectives: Intracytoplasmic Sperm Injection (ICSI) assigns the embryologist with the formidable task of choosing a single normal sperm. In the absence of a reliable test, the choice of the sperm injected into the egg remains arbitrary. However, selection based upon sperm DNA integrity has shown promising results. The Toluidine Blue Assay (TBA) is simple and cost effective. Previous studies using the TUNEL and Comet tests have shown that sperm processed through density gradient centrifugation is enriched with high DNA integrity. The purpose of this study is to demonstrate that the TBA test is efficient in documenting improved sperm DNA integrity following density gradient centrifugation.
Methods: Semen samples were collected from 33 men undergoing ART. Samples were analyzed for DNA fragmentation using the TBA test before and after density gradient centrifugation. Sperm was layered over Enhance-S-Plus media (Conception Technologies, San Diego, CA) and spun at 300g for 20 minutes. Raw and density gradient treated samples were stained for TBA. TBA testing involves application of 0.05% TB, yielding three staining categories: light, medium and dark. In order to enhance this classification, 0.1 % nigrosin was added for background staining. Sperm cells with highly damaged DNA have a greater affinity for the dye, appearing very dark in color, while good chromatin integrity is indicated by practically colorless sperm cells.
Results: Statistical analysis using the Bland-Altman plots demonstrates that for all sperm samples tested by TBA, there was significant improvement of DNA integrity following density gradient processing (Figure 1). In most cases, density gradient samples showed fewer sperm with poor DNA integrity (dark staining).
Conclusions: TBA staining is validated as a reliable method to certify enrichment of a sperm population with high DNA integrity following density gradient centrifugation. Our study confirms that this technique allows for the selection of better sperm with improved DNA quality. In addition, these results further support the clinical usefulness and ease of the bench-top TBA as a test of sperm DNA integrity.
Key Words: Sperm DNA Integrity, Toluidine Blue Assay, Density Gradient Centrifugation



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CHARACTERIZATION OF THE BIOCHEMICAL PROCESSES OF CAPACITATION AND CRYOCAPACITATION AND THE ROLE OF SEMINAL PLASMA IN PORCINE SPERM CELLS

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Capacitation is a biochemical pathway sperm cells undergo to obtain the ability to fertilize an oocyte. Cryocapacitation results from cryopreservation [CP] rendering the sperm cells able to fertilize an oocyte immediately upon thawing. Similarities between functional capacitation and cryocapacitation have not been fully elucidated. Seminal plasma has been demonstrated to attenuate both of these processes. This study attempted to characterize the biochemical pathways of functional

capacitation and artificial cryocapacitation, including the role of seminal plasma [SP] in these events. Merocyanine-540 was used to examine lipid membrane disorder. Lipid disorder increased from 30% of the in vitro capacitated sperm population to 91% in the CP population. In both cases, no change occurred with the addition of 10% SP to the media. Annexin V assessed phosphatidylserine (PS) inversion from the inner to the outer leaflet of the plasma membrane. Cryopreservation resulted in 40% of cells displaying PS on the outer leaflet compared to 13.6% of in vitro capacitated sperm. The addition of SP significantly decreased PS inversion in both populations. Cholesterol efflux was monitored with Filipin; the CP sperm showing a marked increase in efflux (8%) compared to in vitro capacitated sperm (3%). In both in vitro and cryocapacitated sperm, the addition of SP decreased the amount of cholesterol lost from the membrane, 1% and 1.5% respectively. Lipid raft-associated glycolipid GM1 movement was shown to have increased throughout the entire sperm membrane in CP sperm. The addition of SP did not effect lipid raft movement in either sperm population. A late event in capacitation is the phosphorylation of tyrosine residues on membrane proteins. Cryopreserved and in vitro capacitated sperm demonstrated a similar intensity of phosphorylation, with SP reducing intensity. An endpoint examination of capacitation is the ability of the sperm to undergo an induced acrosome reaction (AR). In in vitro capacitated sperm, 67.5% underwent an induced AR with 88.5% reacting in CP sperm. In both cases SP reduced the percentage of AR sperm, 1% and 14.1%, respectively. In conclusion, cryocapacitation appears to initiate freezing-thawing damage to the sperm cell that results in marked increases in membrane disorder, cholesterol efflux, and percent of capacitated sperm. In both capacitated and cryocapacitated sperm, the addition of SP appears to attenuate many of these biochemical processes.

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ROS LEVELS ARE INDEPENDENT OS SPERM CONCENTRATION, MOTILITY AND ABSTINENCE IN NORMAL, HEALTHY PROVEN FERTILE MAN – A LONGUTIDINAL STUDY

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Measurement of ROS level by chemiluminescence method is accurate, easy and reproducible with low intra- and interobserver variability. However, there is a lack of information on correlation of ROS levels with sperm concentration and motility over a period of time within a given individual. We studied the intra-individual variation in seminal ROS level in proven fertile man and its correlation with semen parameters over a course of time.
A single normal healthy proven fertile man provided 38 semen samples over a period of 16 months with an abstinence of 1 to 10 days. Sperm count, motility, leukocytes (Endtz test) and ROS were measured. ROS was measured directly in liquefied semen specimens by chemiluminescence method using luminal as the probe. ROS results were expressed as (X 106 counted photons per min / 20 X 106 sperm). ROS levels >0.0185 were considered as positive.
The mean ± SD for sperm concentration was 106.89 ± 37.77 X 106 / mL and % motility 69.62 ± 10.06. ROS levels ranged from 0 – 0.06. The mean ROS level was 0.0122 ± 0.0141 (95% CI = 0.0169). ROS levels of >0.0185 as seen in 5 of 38 (13%) samples showed no effect on the mean sperm concentration or motility. There was no correlation between the ROS levels and sperm count, motility and abstinence. Leukocytes concentration was 0 in all the samples.
ROS levels within normal, healthy individual show variation over an extended period, however, these levels are independent of concentration, motility and abstinence period. Periodic increase in ROS level might be related to retention of cytoplasm or seasonal variation in spermatogenesis. Our results signify the importance of physiological levels of ROS in semen.

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HUMAN SPERM DNA INTEGRITY: CORRELATION BETWEEN THE TUNEL ASSAY AND A NOVEL TOLUIDINE BLUE ASSAY

Dennis Marchesi, Hannah Biederman, BS, Scott Ferrara, N/A, Huiliang Feng, PhD and Avner Hershlag, MD (Presented By: Dennis Marchesi)
NSLIJ

Objectives: The Toluidine Blue Assay (TBA) has been proposed as a bench-top test to evaluate sperm chromatin integrity, complementing traditional semen analysis. This method is both more cost-effective and time-efficient in comparison to alternative methods, such as the terminal nick-end labeling (TUNEL) assay and the Sperm Chromatin Structure Assay (SCSA). TBA has been positively correlated to semen analysis. The purpose of this study was to compare TBA to the TUNEL test, that some regard as the "gold standard"of DNA integrity testing.
Materials and Methods: Semen samples were collected from 12 men undergoing routine semen analysis. Each sample was analyzed by both the proposed TBA and the TUNEL assay. TUNEL employs a reliable In Situ Cell Death Detection Kit, Fluorescein (Roche Diagnostics, Mannheim, Germany); TBA involves application of a novel, highly specific TB recipe, coupled with background staining to aid in assessment of the slides. Sperm cells with highly damaged DNA have a greater affinity for the dye, appearing very dark in color, while those with good chromatin integrity reject the dye, appearing almost colorless.
Results: Positive correlation was found between TBA and TUNEL. In 10 out of the 12 samples tested, there was a strong association between the two assays with less than 15% error. The overall percentage of sperm with fragmented DNA revealed by the TUNEL assay was similar to that detected by TBA.
Conclusions: TBA is a reliable alternative to TUNEL in the assessment of sperm DNA fragmentation. The ease of the procedure, along with the lower cost and less time required, make TBA an attractive technique that could potentially replace the more cumbersome methods of TUNEL and SCSA.

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RELEVANCE OF MORPHOLOGIC HETEROGENEITY IN HUMAN AND ANIMAL SPERM

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Analysis of morphologic aberrations in human sperm became more important since introduction of assisted reproductive techniques. The aim of our study was to gain new insights in sperm morphology and its relevance for the fertilizing potential by application of differential interference contrast microscopy (DIC), which allows the analysis of non-stained, vital spermatozoa and scanning electron microscopy (REM) in human and animal sperm.
Ten semen specimen of fertile and infertile men as well as of diverse human and non-human primates (bonobo, rhesus monkey, C. jacchus), farm (pig, cattle, rabbit, goat) and laboratory animals (fertile wild type mice, infertile Cyritestin-knockout-mice, fertile DK023 mice with 4 germ cell specific knock outs) were used to evaluate morphology and the number/size of vacuoles of the sperm head by DIC and REM.
An extreme heterogeneity of sperm heads was found in human males. Comparing fertile donors and infertile patients analysis of sperm head morphology revealed inconsistent results without correlation to the fertility status. In contrast, animals showed no or only very few variations of sperm head morphology and no vacuoles with only two exceptions: human primates (bonobo) showed 30% pathomorphologic sperm heads and specimen of the fertile DK023 mice contained up to 80% pathomorphologic sperm heads. Both species and human males had nuclear vacuoles.

The impact of the morphologic aberrations in human sperm including nuclear vacuoles remains speculative. However, the present data suggest rather minor effects.

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COMPARISON OF SPERM CHROMATIN DISPERSION TEST AND TUNEL ASSAYS TO ASSESS THE DNA FRAGMENTATION IN HUMAN SPERM

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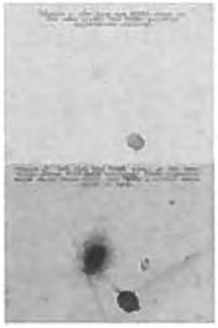
Introduction and objectiveï¼šSperm DNA integrity has been associated with male fertility potential in vivo and in vitro. Sperm DNA fragmentation can be evaluated in a variety of techniques such as TdT-mediated dUTP nick end labeling (TUNEL), sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD), comet assay, and so forth. The SCD test is a recently developed method which could observe using bright-field microscopy in a short period of time. The objective of the research is to compare of SCD test and TUNEL assays to assess the DNA fragmentation in human sperm.
Methods: Semen samples were obtained by masturbation from 10 normozoospermic men who had previously fathered at least one child and 20 idiopathic oligozoospermia (IO) patients after abstinence from sexual activity for 3 days. After liquefaction of semen, standard semen parameters (volume, density, motility, morphology) were obtained according to the World Health Organization guidelines. Sperm morphology was by strict criteria after Diff-Quik staining. The SCD test was performed following the procedure of Fernandez (2003), with some changes in the lysing solution and in test procedure. DNA fragmentation was detected by performing TUNEL assay simultaneously on the same sample slide which was already tested by SCD but still was not stained.
Results: The percent of sperm DNA fragmentation measured by SCD test was significantly higher in infertile patients (34.7%±9.3%) compared to fertile controls (18.1%±3.9%) ($P<0.01$). A positive correlation is detected between SCD test and TUNEL assays ($r=0.876$; $P<0.001$) for detecting sperm DNA fragmentation. Sperm with DNA fragmentation determined by SCD test shows TUNEL-positive when performed TUNEL and SCD on the same sperm.
Conclusions: SCD test could be confirmed by TUNEL assay directly in testing the sperm DNA fragmentation. The presence of sperm DNA fragmentation may lead to male infertility.



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EFFECTS OF VARDENAFIL ON SPERM CAPACITY TO UNDERGO HYPERACTIVATION

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University of Ioannina

Objectives: The outcome of the sperm hyperactivation assay (SHA) is a marker of the sperm fertilizing capacity. We evaluated the role of vardenafil administration in SHA outcome.

Materials and Methods: Each of 42 asthenospermic men produced three semen samples. Each sample was processed for a swim-up procedure and then the recovered fraction of motile spermatozoa was incubated for 8 hours at 37 0C under 5% carbon dioxide in air. Then the percentage of hyperactivated spermatozoa was calculated using a computer-assisted sperm hyperactivation assay system (criteria Burkman, 1991). The above 42 men were then administered 20 mg vardenafil daily for 10 weeks. Then three semen samples were collected from each participant. Semen samples were processed for the same swim-up procedure, and the percentage of hyperactivated spermatozoa was calculated under the above described experimental conditions.
Results: The mean percentage of hyperactivated spermatozoa was significantly larger after vardenafil administration (8.2±1.2) than prior to vardenafil administration (6.0±1.1) ($p<0.05$; Wilcoxon test for paired observations).
Conclusions: Considering that sperm hyperactivation represents a prerequisite for the spermatozoon to obtain ability for fertilization, the increase in the outcome of SHA after vardenafil administration may suggest that spermatozoa collected after vardenafil treatment have higher fertilizing capacity.

FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT

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EVIDENCE FOR THE PARTICIPATION OF HUMAN EPIDIDYMAL PROTEIN CRISP1 IN SPERM-ZONA PELLUCIDA INTERACTION

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Human epididymal CRISP1 (Cysteine Rich Secretory Protein 1), as its rodent counterpart, associates with sperm during maturation and participates in gamete fusion through complementary sites in the oolema. Based on recent observations showing that rodent CRISP1 is also involved in the previous stage of sperm-zona pellucida (ZP) interaction, in the present work we investigated the participation of human CRISP1 (hCRISP1) in this specific step of the fertilization process. For this purpose, human hemizona (HZ) were inseminated with human capacitated sperm in the presence of anti-hCRISP1 polyclonal antibody. As controls, the corresponding HZ were co-incubated with sperm in the presence of normal rabbit IgG or in medium alone (C). Results showed that anti-hCRISP1 produced a significant inhibition in the number of bound sperm per HZ compared to controls (anti-hCRISP1 vs. IgG $p<0.005$; anti-hCRISP1 vs. C $p<0.01$). Insemination of HZ in the presence of bacterially-expressed hCRISP1 (coupled to maltose binding protein, MBP), also produced a significant reduction in the number of sperm bound per HZ compared to HZ incubated in medium alone ($p<0.005$) or medium containing MBP ($p<0.005$). Indirect immunofluorescence experiments using human ZP-intact eggs confirmed the ability of hCRISP1 to bind to the ZP in addition to the reported binding of the protein to the oolema. Finally, with the aim of identifying the ZP ligand of hCRISP1, human recombinant proteins ZP2, ZP3 and ZP4 expressed in insect cells were co-incubated with hCRISP1 or MBP in ELISA experiments. Results revealed that hCRISP1 specifically interacted with ZP3 in a dose-dependent manner. Together, these results indicate that human CRISP1, as its rodent homologue, participates not only in sperm-egg fusion but also in the previous stage of sperm-ZP interaction.

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PARTICIPATION OF EPIDIDYMOSOMES IN THE ASSOCIATION OF EPIDIDYMAL PROTEIN CRISP1 WITH SPERMATOZOA

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Rat protein CRISP1 (Cysteine Rich Secretory Protein 1) associates with the sperm surface during epididymal maturation. Sequential protein extraction experiments revealed the existence of two populations of CRISP1 on epididymal sperm: a major population loosely associated with the cells that is released during capacitation, and a minor, strongly-bound protein, that remains on sperm and participates in fertilization. In spite of its relevance, the mechanisms that anchor the tightly-bound CRISP1 to sperm are still unknown. Recent evidence shows that membranous vesicles from the epididymal fluid named epididymosomes are involved in the association of proteins with the sperm surface during epididymal passage. In view of this, in the present work we investigated the participation of epididymosomes in the transfer of CRISP1 to sperm. For this purpose, epididymal vesicles were obtained by ultracentrifugation of rat epididymal fluid and then examined by electron microscopy to confirm their correct isolation. Western blot analysis using anti-tubulin and anti-CRISP1 as first antibodies revealed the absence of sperm in the epididymosomes preparation and the association of CRISP1 with the vesicles. In order to examine the anchoring of CRISP1 to the epididymosomes, the vesicles were subjected to different protein extraction treatments and the presence of CRISP1 in the protein extract was subsequently analyzed by Western blot. As previously observed for the protein bound to sperm, PBS, 2M NaCl, low pH(3) and 5U/ml PLC-PI (phospholipase C specific for inositol) were not capable of removing CRISP1 from the epididymosomes, while high pH(11), 250mM DTT (dithiothreitol) and 1% Triton X-100 completely extracted the protein from the vesicles. Together, these results confirmed the existence of a population of CRISP1 tightly-bound to the epididymosomes, supporting the participation of these vesicles in the mechanism by which CRISP1 strongly associates with sperm during epididymal transit.

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Originality 5
Methods 5
Present 4
Significance 4

FUNCTIONAL CONSEQUENCES OF CLEAVAGE, DISSOCIATION, AND EXOCYTOTIC RELEASE OF ZP3R/SP56 FROM THE MOUSE SPERM ACROSOMAL MATRIX

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The acrosome is a membrane-bound organelle located in the anterior tip of the sperm head. It is essential for fertilization in mammals. In addition to having different morphological regions, two biochemically distinct compartments can be defined within the acrosome: a particulate acrosomal matrix and a soluble partition. The domains within the acrosome participate in the release of acrosomal proteins from the sperm during exocytosis, depending on whether the proteins partition into either the soluble or matrix compartments of the acrosome. We have examined the mechanism of differential release by evaluating the solubilization of acrosomal matrix protein ZP3R/sp56 from mouse sperm during the course of spontaneous acrosomal exocytosis. Using indirect immunofluorescence and immunoblotting, we found that the ZP3R/sp56 monomer is processed from 67,000 Mr to 43,000 Mr by proteases coincident with release from the acrosome. Sperm require a maturational step termed capacitation before they are competent for acrosomal exocytosis and the processing of ZP3R/sp56 is dramatically reduced under non-capacitating conditions. The cleavage takes place in the bridge region between short consensus repeat numbers 6 and 7, a domain that is not present in the guinea pig orthologue AM67. The cleaved form of ZP3R/sp56 does not bind to unfertilized eggs. We have incorporated these structural considerations into a model to explain the functional consequences of acrosomal exocytosis on sperm-zona interactions.

INFERTILITY / ART / MALE CONTRACEPTION

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O 3
M 3
P 5
S 4

A NOVEL MOUSE MODEL OF ASTHENOSPERMIA

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The Mot1 mouse line was identified in a forward genetics (phenotype to genotype) ENU mutagenesis screen for infertile mouse lines. This mouse line was identified as having male-specific infertility. Using natural matings, Mot1 males are sterile. Sperm were also unable to achieve fertilization via in vitro fertilization. The testes of Mot1 mice display qualitatively normal spermatogenesis at a histological level, however, calculation of daily sperm production shows a 50% reduction compared to fertile Mot1 testes. Computer assisted sperm analysis (CASA) showed that both total sperm motility and progressive motility were significantly reduced when compared to fertile littermates.
The causal mutation was recently identified by using the Affymetrix GeneChip Mouse Mapping 5K SNP kit and subsequent fine mapping and candidate gene sequencing. A point mutation that was homozygous in all infertile Mot1 mice and either heterozygous or wild type in fertile littermates was identified in a previously uncharacterized gene that contains protein domains suggesting an involvement in GTP-binding. GTP-binding is important in intracellular processes such as cytoskeletal organization and protein transport and as such, a breakdown in its normal function could result in abnormal axonemal formation or disrupted intraflagellar transport of proteins which is essential to sperm development and function, thus causing the observed infertility. The A to G transition in the cDNA of the Mot1 gene results in an amino acid change from an acidic and polar aspartic acid to a neutral, nonpolar glycine. This gene is expressed in a wide range of tissues, and appears to be enriched in tissues that are ciliated and thus the presence of other ciliopathies is currently being investigated.
The identification of a mutation causing the Mot1 phenotype has identified a novel regulator of male fertility that is required for sperm development, axoneme function and motility.

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INITIAL SEMEN ANALYSIS WITH AZOOSPERMIA IS PREDICTIVE OF SUCCESSFUL VASECTOMY FOR STERILIZATION

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Objectives: Most urologists request two semen analyses demonstrating azoospermia before advising that the vasectomy is successful. This practice is not evidence-based. We conducted a prospective study to evaluate the effectiveness of initial semen analysis with azoospermia in predicting the success of vasectomy for sterilization.
Methods: A cohort of 108 patients was included in this study. The importance of post-operative semen analysis was emphasized to patients at the time of vasectomy consultation and after the surgery. Vasectomy was performed via a conventional scrotal incision. At least ten ejaculations were required in 6 weeks after the procedure. The first semen analysis was performed 6 weeks after the surgery using the semen analysis techniques recommended by the World Health Organization. The second semen analysis was performed 2 weeks later.

Results: Eighty-six patients (81%) returned for the first semen analysis, all were azoospermic. Forty-three (50%) of these patients had a second semen analysis, and all were azoospermic. Twenty patients (19%) did not return for semen analysis at all. No statistical significance was demonstrated between initial and subsequent semen sample in terms of all semen analysis parameters ($p>0.05$). Seventy (74%) patients had mean follow-up time of 2 years (office visit, mail or phone contact) and all had successful sterilization.
Conclusions: Our data revealed that a second semen analysis is unnecessary after an initial post-vasectomy semen analysis reveals azoospermia. Initial semen analysis with azoospermia is predictive of successful vasectomy for sterilization. Additionally, compliance for post-vasectomy semen analysis is still a significant problem.

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INFERTILITY AND ABNORMAL SPERM IN MICE LACKING THE HIP/RPL29 GENE

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Ribosomal protein L29 (Rpl29), also known as the heparin/heparan sulfate interacting protein (HIP), is a eukaryote-specific ribosomal protein involved in protein synthesis. Previously it has been shown that male mice harboring a loss-of-function mutation in the Hip/Rpl29 gene displayed prenatal growth defects, high mortality rates at birth, distortion of the Mendelian ratio, and at adulthood exhibited a short stature phenotype associated with skeletal fragility and infertility. Here, we investigated the basis for the observed infertility in these mice. Concomitant with reduced body weights in these mice, the testicular weights were also reduced compared to age-matched wild type controls. Restoration of body weights to control levels by high energy diet also restored testicular weights and sperm numbers to that of controls, but did not restore fertility. Testicular histology of the Rpl29 null mice showed normal seminiferous tubules and germ cell associations. The presence of abnormally large numbers of spermatids in the epididymis of null mice, despite normal testicular histology, suggests a critical role for Rpl29, or its dependent genes, in spermiogenesis. When studied at the light microscopic level, the spermatozoa from both the caput and the cauda of these mice were found to be grossly abnormal with defects on the head and tail regions that were not correlated to either diet or body weight changes. The ability of null sperm to undergo the acrosome reaction was significantly reduced ($P<0.01$), compared to controls. However, their ability to bind Rhodamine 123 remained intact suggesting normal mitochondrial potential. Strikingly, a characteristic kinking of the tail was seen in 15-60% of sperm in each sample from null mice, reminiscent of the previously documented, yet uncharacterized 'Dag defect'. The sperm from null mice were also characterized by a 70.5% reduction in progressive sperm motility ($P<0.001$), compared to controls, as well as an absence of hyperactivated motility. These findings, potentiated by the morphological defects, indicate the basis of the infertility of these male mice. Gross defects in both head and tail of the sperm suggest the obstruction of activity of several genes whose efficient expression is dependent on the presence of RpL29 ribosomal protein. Studies are ongoing to elucidate the mechanism involved.

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FIRST REPORT ON THE ABSENCE OF VIRAL LOAD AND USE OF TESTICULAR SPERM SAMPLES OBTAINED FROM MALES WITH HEPATITIS C AND HIV AFTER WASH

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HIV and HCV are sexually transmitted diseases needing a previous treatment of the sperm samples in order to eliminate viral presence and confirm the washing success to be employed in assisted reproduction. Recently, we published a modified sperm wash for severe oligospermic samples. Some azoospermic patients from serodiscordant couples have been referred to our centre recently. Our experience, and recent case reports where epididymal sperm retrieval are presented, leaded us to accept these cases. In this work is to present 3 cases of open testicular biopsies in azoospermic seropositive males for HIV or HCV.
Briefly, open testicular biopsies were carried out with spermatic cord block, opening the scrotal skin and tunica vaginalis, with small incisions through the tunica albuginea to take small pieces of extruding tissue to be carried to the adjacent laboratory for sperm retrieval, in 2 ml of medium and minced mechanically with sterile slides. When sperm cells were found, one half of each sample was submerged in liquid nitrogen for nested PCR determinations (Meseguer et al., 2002), and the other half was frozen following routinely protocols and stored until its employment in assisted reproduction when viral absence is confirmed. Case 1: Male previously fertile, aged 40 years old with a HIV diagnose since 36, no detectable blood viral load, 242 mIU/ml CD4+ cells, and no coinfection. Chronic prostatitis as revealed by echography, caused the obstruction. Normal testes and epididymis TESE yielded 3-4 spermatozoa/field (s/f), 1 motile spermatozoa (ms)/2 f and 1 sperm with progressive motility (spm)/20 f. Case 2: Aged 38 years old, suffered left varicocelectomy at 14. An ejaculation caused by tetrapejia at C4 level two years ago. HCV+ with no blood viral load, treated with interferon 6 years ago. TESE yielded 2-3 s/f, 1 ms/3 f and 1 spm/20 f. Case 3: HCV+ male aged 56 vasectomized 10 years ago. TESE yielded 3-4 s/f, 1 ms/ field and 1 spm/5 f. After sperm washes (Garrido et al. 2008), ms were recovered to be frozen in all testicular biopsies resulting negative for the PCR, then assuring a safe use of the spermatozoa found within the sample. A total number of 4 follicular aspirations, with 3 fresh and 1 frozen/thawed embryo transferences resulting in one pregnancy with a healthy newborn were achieved.
Our results confirm that TESE-ICSI treatments in azoospermic seropositive patients can help them to become fathers safely.

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MOLECULAR PREDICTORS FOR INFERTILITY IN PATIENTS WITH VARICOCELES: A ROLE FOR CYCLIC AMP-RESPONSE ELEMENT MODULATOR (CREM)

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Introduction: We observed a depletion of post-meiotic germ cells and increased apoptosis in testis biopsies from infertile men with varicoceles. These findings were strikingly similar to testis biopsies from CREM-deficient subjects and animal models. Previously, we reported a panel of molecular/genetic markers that related to the pathophysiology of varicoceles and some predicted the poor outcome (<50% increase in post op sperm density) of varicocelectomies (elevated testicular cadmium [>0.453 ng/mg dry wt] and deletions in L-type calcium channel [L-VDCC] exons 6-9).
Objectives: In this study, we investigated the CREM isoforms because they regulated aspects of meiosis. We studied 2 isoforms: a repressor (CREMa) expressed in meiotic cells and an activator (CREMt) expressed post-meiotically, and correlated these isoforms to other markers and the outcome of varicocele surgery.
Methods: Under an IRB protocol, left testis biopsies were collected percutaneously from 31 infertile men at varicocele repair and 5 controls (prior vasectomy) at ICSI. Testis cadmium was assessed by atomic absorption, germ cell apoptosis by TUNEL, and L-VDCC mRNA exons 6-9 (deleted or undeleted) and CREM (present or absent) RNA by RT-PCR. Statistical analyses were performed using the SAS version 9.1.3 software package (SAS Institute, Inc., Cary, NC), with significance set at $P<0.05$.

Results: CREMa and CREMt amplicons were detected in all fertile controls. CREMa was detected in only 58% (18/31) and CREMt was found in 26% (8/31) in infertile men with varicoceles. CREMa correlated with CREMt expression (Fisher's exact test [FET], $p<0.009$). Germ cell apoptosis was elevated in the absence of either CREMa or CREMt (Mann-Whitney tests, respectively $p<0.02$ and $p<0.01$). Absence of CREMa predicted a poor surgical response (FET, $p<0.001$; specificity = 100%; sensitivity = 70%). CREMt expression was not predictive. CREMa absence was associated with deletions in L-VDCC exons 6-9 (FET, $p<0.03$) but was unrelated to biopsy cadmium (FET, $p=0.2$, NS).
Conclusions: This data suggests that absence of CREMa amplicon may be another marker of varicocele repair outcome semi-independent of previous markers, and should be tested further. The CREMa-L-VDCC mRNA association suggests the underlying mechanism of injury may involve cell calcium homeostasis, in agreement with a report that calcium channel blockers deregulate CREM expression (Lee et al., Arch Androl 52:311-8, 2006)
Support: PHS Grant No. ES10496 to SB.

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A NOVEL NON-INVASIVE, MOTILITY-INDEPENDENT SPERM SORTING METHOD AND TECHNOLOGY TO ISOLATE AND RETRIEVE VIABLE SPERM FROM NON-VIABLE SPERM, FOR USE WITH ISCI

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Background and Purpose: Selection of viable sperm for ICSI is challenging and relies primarily upon motility assessment. Current sperm viability assays are limited by subjectivity, limited sensitivity, and irreversible toxicity. Trypan Blue dye exclusion is a gold-standard viability assay. We evaluate the ability of a modified dielectrophoresis (DEP)-based cell-sorting platform to identify and isolate viable sperm from human ejaculate samples containing a natural mixture of viable and non-viable non-motile sperm, pre-incubated with Trypan Blue dye.
Materials and Methods: Fresh ejaculate specimens from 6 men were assessed. Specimen adequacy was confirmed by the presence of motile sperm. Specimens were incubated with 0.4% Trypan Blue and suspended in a sucrose/dextrose isotonic solution. Within 15 minutes, on a custom sorting chip, under 200X magnification, 55 sperm per specimen were assayed: Trypan Blue negative (N=25) and positive (N=25) non-motile sperm, and 5 motile sperm. Trypan stain (+ or -), and whether each sperm was "attracted", "repulsed", or "neutral" to the DEP force, was recorded.
Results: A total of 330 individual sperm were assayed. All (100%) motile sperm visualized in each specimen were Trypan (-), and all those assayed (N=25) were attracted to DEP. All (100%) sperm demonstrating an attractive response to DEP were Trypan (-); none (0%) were Trypan (+). Trypan (+) sperm demonstrated either no response (55%) or a weak repulsive response (45%) to DEP. Some Trypan (-) sperm (12%) demonstrated no response to the DEP.
Conclusions: Results suggest that this modified DEP platform is capable of non-invasively identifying and sorting viable from non-viable sperm. Sorted sperm may be individually retrieved, for use with ICSI. Sensitivity to detect viability may be greater than with the Trypan Blue dye exclusion test.

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THE RARE ADDRA GAZELLE (GAZELLA DAMA RUFICOLLIS) PRODUCES VIABLE SPERMIC EJACULATES THAT TOLERATE CRYOPRESERVATION

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The Addra gazelle (Gazella dama ruficollis) is listed as endangered on the IUCN Red List, largely due to over-hunting and poaching. Fewer than 500 animals of this subspecies remain in the wild, and ~120 individuals are managed in captivity in North American zoos as a research resource and a 'hedge' against extinction. As a first step to developing a self-sustaining, genetically stable ex situ collection, we characterized subspecies seminal traits and then examined sperm sensitivity to cryopreservation. Males (n = 3; 1 ejaculate/male) were subjected to anesthesia and then electroejaculation with semen evaluated for volume, sperm concentration, motility, forward progressive status (scale 0 – 5; 5 = best) and pH. Sperm acrosomal integrity was evaluated using Coomassie blue staining and bright field optics (1000x; 200 sperm/sample). Ejaculates were diluted with an equal volume of TEST Yolk Buffer (TYB Freezing Medium; Irvine Scientific) containing 6% glycerol, loaded in 0.25 ml straws, heat-sealed, cooled to 5°C in a water bath (~3 h), frozen 4 cm over liquid nitrogen for 15 min and plunged into liquid nitrogen. After 1 mo storage, straws were thawed (37°C; 30 s) and sperm suspensions diluted 1:1 with TYB (Refrigeration Medium). Each male produced a spermic ejaculate with quality metrics consistent across males ($P > 0.05$). Mean (± SEM) values for volume, sperm concentration per ejaculate, motility, forward progressive status, spermatozoa with intact acrosomes and pH were: 1.56 ± 0.2 ml, 1.06 ± 0.1 × 109, 58.3 ± 4.4%, 3.0 ± 0.0, 92.5 ± 1.8% and 8.2 ± 0.1, respectively. Sperm motility (38.3 ± 9.3%) and proportions of spermatozoa with intact acrosomes (83.7 ± 2.9%) were lower ($P < 0.05$) after thawing. Incubation at 37°C for 3 h caused no further decline ($P > 0.05$) in sperm motility (26.7 ± 14.5%) or acrosomal integrity (80.8 ± 4.3%). Results indicate that specimens of the rare Addra gazelle produce good quality ejaculates retaining mostly motile spermatozoa with high proportions of intact acrosomes. Furthermore, the immediate post-thaw survival of these sperm to a standard cryopreservation protocol falls within the range of earlier studied and more common wild ungulates (including other gazelle species). These data lay a foundation for more detailed investigations to enhance both natural and assisted breeding to develop a sustaining ex situ population of this endangered subspecies. (Funded by Sichel Endowment Fund and Friends of the National Zoo)

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VASECTOMY BY EPITHELIAL CURETTAGE WITHOUT SUTURE OR CAUTERY: A PILOT STUDY IN MAN

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Background: Vasectomy with suture ligation and/or cautery of the vas deferens is a very effective form of male sterilization; however, the procedure can occasionally fail or cause chronic testicular pain. Curettage of the epithelium of the vas might allow for effective male sterilization while minimizing tissue destruction, thereby leading to a lower incidence of testicular pain. In addition, epithelial curettage might simplify the procedure by obviating the need for electrocautery or suture.
Study Design: We conducted a pilot study of vasectomy by epithelial curettage with a novel microcurette called the Vas-X in twelve normal men requesting elective sterilization. Seminal fluid analysis was performed monthly after the procedure. Procedural and post-operative pain was assessed by questionnaire.

Results: All twelve men tolerated the procedure without complications. Sperm concentration decreased dramatically within the first month in 11 men (99.7 million/ml vs. 0.9 million/ml; $p < 0.001$), but remained elevated (48 million/ml) in one subject. Ten men attained severe oligospermia (<100,000 sperm/ml) by three months; however one subject's concentration increased to 4 million/ml at four months after achieving severe oligospermia at month three. Follow-up is ongoing. Post-operative pain was minimal and all men reported normal sexual function post-operatively.
Conclusions: Vasectomy by epithelial curettage with the Vas-X is an effective form of male sterilization in most of the men in this small pilot study. However, two of the subjects were not effectively sterilized by the procedure. Epithelial curettage will require further refinement to determine if it is a viable alternative to cautery for vasectomy provision.

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THE HYALURONAN BINDING ASSAY (HBA): A STATISTICALLY SIGNIFICANT AND ROBUST DIAGNOSTIC ASSAY IN DIRECTING ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) TREATMENT AND PREDICTING CLINICAL PREGNANCY (CP)

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Introduction and Objectives: Male factor infertility (MFI) serves as the underlying etiology in 40% of the infertile couples attempting conception. Established andrology assays assist in directing the clinician towards the selection of the optimal ART treatment. The HBA assay assesses the ability of the seminal population to bind to an immobilized substrate of hyaluronan. Hyaluronan-bound (HB) sperm carry reduced levels of chromosomal aberrations, enhanced levels of developmental maturity and are associated with higher proportions of normal head morphology. These relationships led to the current study examining specific assays which serve to evaluate common MFI parameters and their individual contribution to outcome. The study explored the relationship between the HBA index, Kruger Strict Criteria (KSC) morphological assessment, total number of motile sperm (MS), hemizona assay index (HZA) and the likelihood of CP.
Methods: A retrospective analysis of 253 infertile couples examined each MFI parameter as to its ability to direct the ART treatment and contribute to a CP. Data collection followed an IRB-approved protocol. With the CP as the dependent variable and the HBA index, KSC, MS, and HZA as the independent variables, multivariate linear regression and Pearson's correlation were used to analyze the data with SPSS 15.0.
Results Obtained: Multivariate linear regression confirmed the MFI parameters correlated with the HBA index ($R2=.596$). Logistic regression showed that the HBA index played a statistically significant role in contributing to the likelihood of CP ($p=.003$) adjusting for ART method and mother's age. KSC, MS and HZA demonstrated no statistical significance ($p>0.05$) in their ability to contribute to CP.
Conclusions: The current study illustrates that the HBA index, adjusting for ART method and mother's age, is a statistically significant indicator of CP. In contrast, assessment of the seminal population by KSC, MS and HZA did not carry statistical significance in their individual or combined ability to predict CP. Although its value correlated with the seminal parameters described by KSC, MS and HZA, the HBA index served as a more robust indicator of clinical success. The data demonstrates that the use of the HBA assay in the evaluation of the infertile couple merits strong consideration when selecting the most appropriate ART treatment to achieve CP.

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EMBOLIZATION VERSUS MICROSURGICAL VARICOCELECTOMY FOR TREATMENT OF CLINICAL VARICOCELES: SHORT-TERM OUTCOMES AND IMPLICATIONS FOR IVF/ICSI

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Introduction and Objective: Treatment of varicoceles in infertile men with either transvenous embolization (TE) or microsurgical varicocelectomy (MV) has shown similar long term benefits on semen parameters and fertility rates. However, surgery may have detrimental effects on spermatogenesis in the short term, and this may be important for couples who are considering the use of intra-uterine insemination (IUI) or in-vitro fertilization (IVF/ICSI) in the immediate post-operative period. We compared early post-treatment outcomes in infertile patients undergoing either TE or MV for treatment of clinical varicoceles.
Methods: We retrospectively reviewed records of men who had undergone either TE or MV for treatment of a clinically palpable varicocele associated with male infertility. Semen analyses performed prior to treatment and 3- and 6-months after treatment were compared. The primary outcome was a change in the total motile sperm count (TMC = ejaculate volume (mL) x concentration of motile sperm). Within- and between-group comparisons were made using paired and independent T-tests, respectively.
Results: 56 patients were evaluated, with 28 patients in each group. Baseline TMC was similar for the TE (12.1 +/-23.1 million) and MV (11.6 +/- 15.5 million) groups. At 3 months there was a significant improvement in the TE group (TMC increased by 301% +/- 582%, $p=0.036$) but not in the MV group (TMC increased by 81% +/- 233%). At 6 months, both the TE and MV groups showed significant improvements in TMC from baseline. No significant differences in degree of improvement in TMC between the two groups was identified after 6 months of follow-up. 3 patients (11%) in the MV group became temporarily azoospermic following treatment, compared with none in the TE group.
Conclusion: In this series, TE showed superior short-term (3 month) improvements in TMC when compared to patients treated with microsurgical varicocelectomy. Transient negative effects of anesthesia and surgical trauma on spermatogenesis possible explain such effects. Importantly, 3 patients who underwent surgery became azoospermic after treatment. These findings must be taken into account when counseling patients who are considering undergoing IUI or IVF/ICSI shortly following varicocele treatment.

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INFLUENCE OF SPERM CRYOPRESERVATION ON THE OOCYTE ACTIVATING FACTOR PLCÆ

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Introduction: Cryopreservation of human sperm is considered a routine procedure in ART. However, cryodamage to acrosomal structures, nuclei and cytoplasmic integrity has been described and it has been suggested that fertilization capacity is decreased after freezing-thawing. There is now substantial evidence to suggest that fertilization is triggered by PLCæ. The amount of PLCæ introduced in the oocyte is critical for activation and also for subsequent embryo development. We set out to study if the oocyte activating factor is decreased in human sperm cells after cryopreservation.

Methods: Human ejaculated spermatozoa were obtained form the consenting patients without male infertility who attended our center for participation in an IVF program. After density centrifugation an aliquot was used for protein extraction. To extract protein from the motile sperm cells a swim up was performed. Freezing was performed by manual cooling in cryoprotection buffer (Spermfreeze, FertiPro) according to instructions provided by the manufacturer. The samples were thawed by fast warming to room temperature and washed in gamete buffer (Cook). Immunoblotting was performed using polyclonal anti-peptide antibodies generated against human PLCæ sequences and immunoreactive bands were visualized with the enhanced chemiluminescence detection system. Relative average densities (rel.int.) of the bands were statistically compared with t-test.
Results: Quantity of protein extracted was not significantly different between fresh samples (n=5) and cryopreserved samples (n=5) (2.4±0.9 and 2.5±0.6 µg/106 sperm cells respectively; p>0,05). Intensity of the band representing the full length PLCæ protein of the sperm samples after cryopreservation was decreased in comparison with the samples before freezing (rel.int. 0.30±0.03 versus 1.00±0.27, p<0.01). Extraction and blotting of proteins from the motile fraction of the sperm samples showed that the intensity of the PLCæ band was reduced 50% after freezing in comparison with the motile fraction form non-cryopreserved samples (p<0.01).
Conclusions: The oocyte activating factor PLCæ is decreased after freezing/thawing of sperm samples, which may lead to reduced fertilization. This is probably due to a decrease in membrane integrity and the consequent increased membrane leakage. Sperm freezing medium containing egg yolk would be protective for cryodamage on the membrane, and a study on PLCæ comparing the two sperm freezing media is ongoing.

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EFFECT OF THE AGE OF THE MALE ON PREGNANCY RATES ADJUSTED FOR ABNORMAL HYPO-OSMOTIC SWELLING TESTS USING A DONOR EGG MODEL

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Introduction and Objectives: The risk of subnormal hypo-osmotic swelling (HOS) test scores <50% may increase with advancing male age. This abnormality leads to severe embryo implantation defects but this problem can be overcome by fertilizing the eggs with intracytoplasmic sperm injection (ICSI). The purpose of the present study was to evaluate the effect of the age of males on fecundity eliminating the female factor by using younger donated oocytes but also uniquely adjusting for low HOS scores by performing ICSI when HOS was <50% even if other semen parameters were normal.
Methods: The clinical (viable fetus at 8 weeks), live first trimester, and live delivered pregnancy rates and implantation of all donor egg recipient cycles were evaluated according to the age of the male partner. There were 6 age groups: 1) <35, 2) 36-40, 3) 41-45, 4) 46-50, 5) 51-55, 6) >56.
Results obtained: The clinical pregnancy rates in the 6 groups were 54.8% (86/157), 56.0% (107/191), 60.3% (129/214), 50.4% (67/133), 59.2% (29/49), and 56.0% (14/25)). The first trimester live rates were 50.3%, 50.8%, 51.9%, 42.9%, 53.1%, and 52.0% and the live delivered pregnancy rates were: 48.4%, 48.2%, 48.6%, 35.3%, 46.9%, and 52.0%. The implantation rates were 30.2% (146/180), 30.5% (163/534), 34.0% (206/605), 26.3% (97/369), 34.3% (46/134), and 37.1% (26/70).
Conclusions: There was no trend for lower rates of achieving pregnancies or higher rates of miscarriage in the female partners of males with more advanced age when ICSI was used to adjust for low HOS test scores.

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EFFECT OF LUBRICANTS DEVELOPED FOR FERTILITY MARKETS ON IN VITRO FERTILIZATION AND EMBRYO DEVELOPMENT

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Introduction: Traditional lubricants damage sperm and should not be used when pregnancy is desired. Newer products have been developed for this consumer/patient group. Bovine and human embryos share similar paternal sperm regulatory pathways, making this species a model for detection of sublethal sperm damage (RepBioMedOn 2002;4:170).
Objective: Experiments were done to evaluate in vitro fertilization and embryo development following bull sperm exposure to lubricants developed for the fertility market.
Methods: Cryopreserved bull sperm was washed, resuspended in medium and placed into one of 5 treatments. These were: 1) Control medium; 2) Pré® Lubricant (Pré); 3) FertilityCare™ (FC); 4) ConceiveEase™ (CE) or 5) PREConceive plus™ (PC). Lubricants were mixed with sperm at 10% v/v and incubated for 30 min at body temperature. Then sperm from each treatment was placed into fertilization wells with mature bovine oocytes. At 8 hr, putative zygotes were transferred into development medium and further incubated. At 32 hr of culture, dividing embryos were counted (% fertilized oocytes).
Results: Embryo development (%) was determined by the number of morulae and blastocysts on Day 7. Friedman's test compared percent fertilization and embryo development in each treatment versus control.
Conclusion: Sperm contact with Pré did not interfere with fertilization or embryo development, whereas other lubricants caused declines in these end points. The reasons for these differences require additional study.
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PREConceive plus™, Lake Consumer Products, Inc., Jackson, WI

Vitro Fertilization & Embryo Development

Treatment	Total Oocytes	% Fertilized Oocytes (±sd)	% Embryos Developing (±sd)	p value fertilization / developed
Control	160	53(8)	45(12)	
Pré	160	52(11)	42(5)	0.500 / 0.470
FC	160	53(12)*	28(20)*	0.003 / <0.0001
CE	160	56(9)*	27(18)*	0.033 / <0.0001
PC	160	56(10)*	26(16)*	0.025 / <0.0001

*notes treatment means differ from control

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THE EFFECT OF GONADOTROPINS ON BOVINE OOCYTES MATURATION IN VITRO: ANIMAL MODEL

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To determine the impact of gonadotropins on oocyte maturation in vitro. A total of 1600 bovine cumulus-oocytes complexes (COCs) were purchased commercially and matured in TCM-199 with 10% FBS and gonadotropins (0, 5, 10, 20, 40, 80 IU/mL) and HCG (0, 5, 10, 20, 40USP/ml). In Exp.1: Bravelle only; Exp.2: Luveris only; Exp. 3: Menopur only; Exp.4: Repronex only; Exp.5: Norvarel only; Exp.6: Menupur + Bravelle; Exp.7: Repronex + Bravelle; Exp.8: Bravelle + Norvarel. 200 oocytes were used for each group. 10 oocytes were exposed to each dilution. Each experiment was repeated twice. All oocytes were cultured at 39 oC, 5% CO2. After 24 and 48 hrs, cumulus expansion was estimated under dissecting microscope. Oocyte maturation rates were determined nuclear maturation (GV, MI, MII) and polar body formation under inverted microscope. Statistics utilized the Fisher's exact test. The results suggested that culture media supplemented with Bravelle, Luveris, Menopur and Repronex significantly improved COCs expansion after 24

hours. HCG only improved COCs expansion at high concentrations. All gonadotropins can enhance the oocyte maturation in vitro in a dose-response manner. The maturation rates after culture for 24 / 48 hours at 20 IU/mL were 13.1 / 45.0 % (B), 33.3 / 37.5% (L), 50.0 / 71.4% (M), 53.8 / 81.3% (R) and 37.5 / 44.4% (N), respectively. In combination, the biological concentration was 10 IU/mL in M+B, R+B and B + N, respectively. Oocyte maturation rates after culture for 24 / 48 hours were 37.9 / 66.7% (M+B), 50.0 / 73.9%(R+B), 52.4 / 75.0%(B+N), respectively. The results also suggested that high concentration of gonadotropins(R or N at 40 IU/mL; R+B and N+B at 20 IU/mL) can result in maturation arrest. In summary, all gonadotropins enhance oocyte maturation in vitro in a dose-response manner. Alone, Menopur and Repronex are more powerful in promoting oocyte maturation; in combination, all of them effectively enhance maturation. However, high concentrations of gonadotropins might result in maturation arrest. The results of this study could provide guidance as to optimal gonadotropin stimulation protocols for both IVF and non-IVF cycles. (Ferring Pharmaceuticals Academic Research Grant).

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THE FREQUENCY OF SUBNORMAL HYPO-OSMOTIC SWELLING TESTS INCREASE WITH ADVANCING AGE OF THE MALE

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Introduction and Objectives: Males with low hypo-osmotic swelling (HOS) scores (<50%) rarely achieve pregnancy after natural intercourse or intrauterine sperm injection. Sperm with low HOS scores fertilize oocytes at a normal rate; however, the embryos formed have extremely low odds of implanting. The present study evaluated whether there was a correlation between this abnormality and the age of the male.
Methods: A retrospective review was made of the first semen analyses of male partners whose wives were infertile. They were sorted into the following age groups: <29.9 years, 30.0-34.9 years, 35.0-39.9 years, 40.0-44.9 years, 45.0-49.9 years and >50.0. A hypo-osmotic swelling test was performed on each specimen.
Results obtained: A total of 4309 patients were evaluated in this retrospective study. The respective percentages for low HOS scores within these age groups are as follows: 26/481 (5.41%), 77/1173 (6.56%), 103/1288 (8.00%), 81/835 (9.70%), 46/357 (12.89%), and 44/175 (25.14%).
Conclusions: There is a clear upward trend in the chance of a male having a low HOS as he gets older. For males at the age of 50 and above, there is a 25% chance of having a low HOS test score. Though only <7% of males <35 years of age have this abnormality, it would be devastating for this group to learn that they have wasted time and money especially if IVF was performed with conventional oocyte insemination when intracytoplasmic sperm injection completely corrects the problem.

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THE EFFECT OF THE LACK OF SPERM WITH RAPID LINEAR PROGRESSION ON PREGNANCY RATES FOLLOWING IN VITRO FERTILIZATION WITH INTRACYTOPLASMIC SPERM INJECTION

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Introduction and Objectives: Previous studies have found a very poor outcome following in vitro fertilization-embryo transfer (IVF-ET) with conventional oocyte insemination if the pre-washed and post-washed specimen failed to have any sperm with rapid linear progression. The results, however, were reasonable if the post-washed specimen showed some sperm with rapid linear progression. The objective of the present study would be to see how these parameters effect outcome with IVF-ET with intracytoplasmic sperm injection (ICSI).
Methods: All IVF cycles were identified where there was fertilization of retrieved oocytes by ICSI with sperm in the pre-washed sample without rapid linear progressive motion. The percentage of retrievals resulting in no embryos to transfer and the fertilization rates according to whether the post-washed specimen had any sperm with rapid linear progression were not determined.
Results obtained: The majority of males with 0% rapid linear progression pre-wash show 0% on post-wash (456/741, 61.5%). The percent with failed fertilization was 4.6% with 0% post-wash vs. 5.6% (p=NS). The fertilization rates did not show any difference (61.9% vs. 64.9%). There were significant differences found in the 8 week and 12 week viable pregnancy rates (8 week 39.1%, 109/279 vs. 48.6% 89/183, p=0.05) and 12 weeks 34.8%, 97/279 vs. 45.4%, 83/183, p=.03).
Conclusions: In contrast to conventional oocyte insemination ICSI markedly improves IVF outcome when faced with post-washed sperm without rapid linear progressive motion. However the pregnancy rates are lower than sperm with 0% pre-wash showing some sperm with linear progressive motion on post-coital.

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LOW HYPO-OSMOTIC SWELLING TEST SCORES CORRELATE BETTER WITH LOWER PERCENT MOTILITY THAN ANY OTHER ABNORMAL SEMEN PARAMETERS

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Introduction and Objectives: Men with low hypo-osmotic swelling (HOS) scores (<50%) rarely achieve pregnancy after natural intercourse or intrauterine sperm injection. Sperm with low HOS scores fertilize oocytes at a normal rate, however, the embryos formed have extremely low odds of implanting. The objective was to find if low HOS scores are more closely associated with certain abnormal semen parameter(s) in comparison to others.
Methods: A retrospective review of first semen analyses of the male partner of infertile couples was performed. The semen parameters evaluated included sperm concentration <20x106 sperm/mL, strict normal morphology <5% and <2%, percent motility, and anti-sperm antibodies. The sperm was analyzed according to the percentage of subnormal HOS tests (<50%) in association with other single subnormal sperm parameters.
Results obtained: A subnormal HOS score was found in 1.9% (n=212) of males with a single concentration defect, 4.52% with strict morphology <5% defect and 3.51% with the subset <2% strict morphology (n=487 and 57), 5.30% with anti-sperm antibodies >50% (n=132), but was 14.22% with motility <50% (n=443). The worse the percent motility, the higher the percentage of patients with low HOS score (33.3% for 0-19%, 30.0% for 20.0-29.9%, 24.10% for 30.0-39.9%, and 10.27% for 40.0-49.9%). The majority of males (331/443) had a percent motility between 40.0-49.9%).
Conclusions: Though low HOS scores may exist without any other semen abnormality, if it is associated with any defective parameter it is more likely with low percent motility.

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THE MAJORITY OF MALES WITH SUBNORMAL HYPO-OSMOTIC TEST SCORES HAVE NORMAL VIABILITY

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Introduction and Objectives: Men with low hypo-osmotic swelling (HOS) scores (<50%) rarely achieve pregnancy after natural intercourse or intrauterine sperm injection. Sperm with low HOS scores fertilize oocytes at a normal rate, however, the embryos formed have extremely low odds of implanting. The HOS test measures the functional integrity of the sperm membrane whereas viability measures the structural integrity. Nevertheless, some andrologists believe that if you measure viability and it is normal, then there is no reason to perform the HOS test. The objective of this study was to determine the percentage of patients with subnormal HOS scores that have subnormal viability.
Methods: A retrospective review was performed. The data only accounted for initial semen analyses. A subset of those males with HOS scores <50% were identified. The viability was then evaluated.
Results obtained: There were 361 males with low HOS scores. Only 12.47% (45/361) of these males had a subnormal viability.
Conclusions: These data show that if centers decided to only test viability and not HOS, then almost 88% of males with problematic low HOS scores would be overlooked. It is clear that viability and HOS tests measure different entities with only a small overlap. All patients with subnormal viability will have subnormal HOS tests, but the converse is not true.

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TENDENCIES IN THE CLINICAL INDICATIONS OF SPERM DONATION OVER THE LAST 10 YEARS AND ITS EFFECTS ON REPRODUCTIVE OUTCOME

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Introduction: Our aim is to study the indication and outcome of semen bank and its evolution in the last 10 years alongside with the development of new techniques in ART as well as changes in the social needs.
Materials and Methods: we retrospectively analyzed our sperm donation cycles (artificial insemination (AI) and IVF) between 2000 and 2007. A total of 2934 cycles were analyzed. We compare by an stratified logistic regression analysis the reproductive outcome depending on sperm donation clinical indications together with their evolutions by trend tests.
Results: the stratified trend test results demonstrated in women without couple the absence of tendency from 2000 until now in AI but a clear positive tendency in IVF cylces (ï•£M-H=-7.276 p<0.001), . In azoospermic patients we perceive a negative tendency in AI(ï•£M-H=-3.003 p=0.003) and IVF (ï•£M-H=—2.070 p=0.039). In IVF/ICSI failure patients we did not distinguish any positive tendency in AI; nevertheless, we detect also a clear negative trend in IVF (ï•£M-H=-4.670 p<0.001). Regarding pregnancy rates we got a model adjusted by AI or IVF procedure performed in which we concluded that indication of azospermia (OR= 1.396 (CI 95% 1.130-1.794)) and IVF/ICSI failure indication (OR= 1.698 (CI 95% 1.388-2.079)) presented higher pregnancy chances than single women without male partner. When we analyzed each indication by separate we observed clearly that the mean age of women without couple is significantly higher than the rest indications (IVF/ICSI failures and azoospermia).
Conclusion: donor sperm bank using has suffered an evolution in the last years, increasing in single women users and decreasing azoospermia and ART failure procedures. Unfortunately, single women had fewer chances to get pregnant, forming a sub-fertile women population. Factors associated with this subfertility should be related with advanced maternal age.

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ABOUT 13% OF WOMEN MAY HAVE THE WRONG METHOD OF OOCYTE INSEMINATION WHEN UNDERGOING IN VITRO FERTILIZATION BY FAILURE TO EVALUATE THE ABNORMAL HYPO-OSMOTIC SWELLING TEST SCORE

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Introduction and Objective: Some studies demonstrate that embryos formed from sperm with low hypo-osmotic swelling (HOS) tests look normal but fail to implant if fertilized by conventional oocyte insemination but is corrected by intracytoplasmic sperm injection (ICSI). Most in vitro fertilization (IVF) centers do not measure this abnormality. The objective of the present study was to determine what percentage of IVF-embryo transfer (ET) might fail to achieve a pregnancy by not performing ICSI because of ignorance of the HOS abnormality.
Methods: Sperm concentration, percent motility, percent normal morphology using strict criteria, antisperm antibodies by direct immunobead testing and HOS tests were performed on semen specimens prior to IVF-ET. The assumption was made that most IVF certain would not perform ICSI for motile densities >8x106/mL, strict normal morphology >4% and absence of antisperm antibodies.
Results Obtained: For women age <39, the frequency of low HOS scores (<50%) was 12.4% (206/1663) had subnormal HOS scores <50% (13.3% (44/330) for husbands of women aged 40-42. The clinical pregnancy rates were 35.2% (19/54) for the younger group and 35.7% (5/14) for the older group. The live delivered pregnancy rates were 33.3% (18/54) and 21.4% (3/14).
Conclusions: These data reconfirm previous studies showing that fertilization by ICSI for sperm with low HOS tests provide reasonable live delivered pregnancy rates. Other previous studies had found almost no live pregnancies with conventional insemination. These data suggest that without performing this simple inexpensive test a significant minority of women (about 12.5%) may have repeated implantation failures by failing to do ICSI related to this occult abnormality.

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EFFECT OF METHOD OF OOCYTE FERTILIZATION ON FERTILIZATION, PREGNANCY AND IMPLANTATION RATES IN WOMEN WITH UNEXPLAINED INFERTILITY

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Introduction and Objective: There are data suggesting that fertilization of oocytes with intracytoplasmic sperm injection (ICSI) when used for sperm with subnormal morphology may result in lower pregnancy and implantation rates than when fertilized by conventional oocyte insemination. The objective of this study was to determine in cases of unexplained infertility whether conventional oocyte insemination vs. intracytoplasmic sperm injection results in differences in fertilization rates, frequency of failed fertilization, clinical and live delivered pregnancy rates, and implantation rates.
Methods: Retrospective evaluation of these parameters in couples undergoing in vitro fertilization embryo transfer (IVF-ET) (minimum 2 embryos) in women with unexplained infertility over a 7-year period.
Results obtained: There was a significantly higher fertilization rate (p<.001) with ICSI vs. conventional insemination (73.7% vs. 63.7%). However of greater clinical importance, the clinical and live delivered pregnancy rates were significantly higher with conventional insemination (52.7% and 46.2%) than with ICSI 33.6% and 29.0%. The implantation rates were also significantly higher with conventional oocyte insemination (24.9% vs. 17.8%). Failed fertilization were low in both groups.

Conclusions: The process of ICSI whether it involves possible subtle oocyte damage by the procedure or the andrologist not choosing the ideal sperm may lead to embryo that are less hearty despite their normal appearance.

EPIDIDYMIS / VAS DEFERENS / SEMINAL VESICLES

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PERK/DUSP6 HAVE REGULATORY ROLES IN ESTABLISHING AND MAINTAINING THE INITIAL SEGMENT OF THE EPIDIDYMIS

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Many of the functions of the initial segment (IS), a region of the epididymis important for male fertility, are regulated by testicular luminal fluid factors through a process known as Lumicrine Regulation. The long-term goal of our studies is to understand the mechanisms of this type of regulation. The focus of this study is to examine the hypothesis that extracellular signal-regulated kinase (ERK) and dual specificity phosphatase 6 (dusp6) respond to lumincrine factors, then participate in a diverse range of cellular responses affecting proliferation, differentiation and protection from apoptosis in the IS. Very low levels of pERK and dusp6 mRNA were observed in the IS during embryonic and postnatal (P) period until P17, at which time, levels of both increased significantly in the IS. Following the surge of pERK/dusp6 mRNA, the height of the IS epithelium increased and the cells began to undergo differentiation. Cell proliferation continued until P40. When efferent duct ligation (EDL), which blocks the testicular fluid from entering the epididymis, was performed on P21 juvenile mice, a reduction in pERK/dusp6 mRNA levels as well as a reduction of cell proliferation and differentiation in the IS was observed. EDL on adult mice also resulted in a reduction of pERK/dusp6 mRNA levels, however, the cells underwent a wave of mass-apoptosis, a phenomena previously reported by a number of investigators. In the dusp6 null mice, epididymal cells continued to undergo proliferation beyond P40 resulting in an increase in epididymal size and weight compared to wild-type controls, confirming the regulatory role of dusp6 in epididymal cell proliferation. Our data suggest that high pERK/dusp6 activity in the IS is sustained by the lumicrine factors, which in tum are required for establishing normal epididymal development, as well as maintaining normal epididymal function in the adult. Supported by NIH-NICHD Grant HD052035.

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VASECTOMY INFLUENCES EXPRESSION OF CRISP1 IN HUMAN EXCURRENT DUCT

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The CRISP family is a group of proteins characterized by two conserved domains and a set of 16 conserved cysteine residues. In mammals, Cysteine-rich secretory protein 1 (CRISP1), a major glycoprotein secreted by the epididymis, is involved in gamete interactions. The epididymis is essential for the acquisition of sperm fertilizing ability and forward motility. Considering that, after vasectomy, the flux and composition of the epididymal fluid are modified, the epididymis can be exposed to sequelae under vas deferens occlusion performed during vasectomy procedures. Some of these sequelae may not be reversible following vasovasostomy, affecting sperm physiology and their fertilizing ability. We have previously demonstrated that the amount of CRISP1 mRNA and protein expressed in the human epididymis are affected under vasectomy. While synthesized in the corpus and cauda epididymidis, CRISP1 accumulates in the cauda region under vas deferens occlusion. In this study we used an antibody raised against CRISP1 to localize this protein in the epididymis. Following vasectomy, the expression level of CRISP1 increased dramatically in the lumen of the cauda epididymidis. In situ hybridization showed that CRISP1 transcript is expressed in the principal cells of the corpus and cauda epididymidis and this localization was not affected by the vasec Western blots analyses were performed to determine the amour CRISP1 on spermatozoa of men who had undergone surgical va reversal. Many vasovasostomised men are characterized by high CRISP1 levels on spermatozoa when compared to normal donors. There was no linear correlation between CRISP1 levels and the period of time elapsed between vasectomy and vasovasostomy. CRISP1 was also present in seminal plasma of normal and vasovasostomised men, but not in vasectomised individuals. CRISP1 was immunodetectable at a higher level in vasovasostomised men when compare to seminal plasma of normal men. Our results demonstrate that the epididymides are the contributors of CRISP1 in human seminal plasma and unusual high levels of this protein associated to spermatozoa of vasovasostomised men may reflect epididymal sequelae occurring when the vas deferens are obstructed. This research was supported by CIHR-Canada grant to RS.

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THE IDENTIFICATION OF MOUSE SPERM PROTEINS THAT EXHIBIT DIFFERENTIAL PATTERNING BETWEEN CAPUT AND CAUDA EPIDIDYMAL SPERM

Takashi Ijiri, PhD, Wenlei Cao, MD and George Gerton, PhD (Presented By: Takashi Ijiri)
University of Pennsylvania

After sperm leave the testis, they undergo biochemical and physiological modifications in the epididymis. As a result, sperm acquire motility and the ability to fertilize eggs. In these modifications, we focused on the addition and elimination of sperm proteins during epididymal transit to understand the molecular mechanisms of sperm maturation. We are using a large-scale proteomic approach to identify proteins of caput and cauda epididymal sperm that exhibit differences in amounts or positions using two-dimensional fluorescence difference gel electrophoresis (2-D DIGE). Protein spots (388) were selected statistically ($p < 0.01$) in analytical gels and 21 and 15 spots were identified as the candidates for relatively differential proteins in caput epididymal sperm and cauda epididymal sperm, respectively. Of these, 16 and 10 spots could be recovered following preparative gel electrophoresis and submitted for protein identification. Of 8 caput epididymal sperm-differential proteins, 5 seem to be concerned for assembly of sperm components. Three of them are molecular chaperones. Two, actin and sperm equatorial segment protein, are structural and cytoskeletal proteins. In 9 cauda epididymal sperm-differential proteins, 5 are important for producing energy for sperm motility. Four of them: aldolase 1, alpha enolase, pyruvate dehydrogenase E1 beta subunit (PDH-E1beta) and triose-phosphate isomerase (TPI) are enzymes of glycolytic pathway. Also mitochondrial ATP synthase O subunit plays a role for ATP synthesis. Western blotting was performed to validate the 2-D DIGE results. The amounts of each of these 3 molecular chaperones and actin from caput epididymal sperm-differential proteins were more abundant in caput epididymal sperm. In these 4 glycolytic enzymes from cauda epididymal sperm-differential proteins, alpha enolase and PDH-E1beta were slightly more abundant in cauda epididymal sperm; however, aldolase 1 and TPI did not confirm the 2-D DIGE results. Our results suggest that most caput epididymal sperm-differential proteins may contribute to the rearrangement of sperm structures and some cauda epididymal sperm-differential proteins may be involved in an increase in ATP production that promotes sperm motility. Supported in part by NIH grants P01HD-06274, T32HD007305-22, ES013508-02, and HD051999.

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EXPRESSION OF EPIDERMAL GROWTH FACTOR (EGF) AND ITS RECEPTOR (EGFR) ALONG RAT EPIDIDYMIS AND ISOLATED SPERM

Marilia Patrao, Acacio Silveira-Neto, Graduated and Maria Christina Avellar, Professor (Presented By: Marilia Patrao)
UNIFESP-EPM

Introduction: EGFR activation is involved in spermatogenesis, acrosome reaction and regulation of ejaculated sperm motility. The role of EGF and EGFR in epididymal physiology and sperm maturation, however, is not known. In this work, EGF and EGFR expression in rat epididymis and maturing sperm was evaluated. The influence of testicular factors on epididymal EGF and EGFR expression was also analyzed using bilateral efferent ductules ligation (EDL).
Methods: EGF and EGFR expression in adult Wistar rat epididymis was assessed by Western blot (WB) and immunohistochemistry (IHC), using anti-EGF, anti-EGFR and anti-pEGFR antibodies. Proper negative controls (blocking peptides) were used. EGFR activation was analyzed by WB in epididymis stimulated in vitro by EGF (100 ng/ml; 1-15 min). RT-PCR with total RNA from initial segment (IS), caput (CP), corpus (CO) and cauda (CD) from rats submitted or not to bilateral EDL (15 days) was performed with primers against Egf and Egfr. EGF, EGFR and pEGFR immunodistribution was also investigated in these groups. EGF and EGFR expression was tested on sperm from testis (T), IS+CP, CO and CD by immunofluorescence (IF).
Results: WB indicated that EGF (precursor and mature forms) and EGFR (total and phosphorylated receptor) are expressed in the rat epididymis. pEGFR basal levels were increased by in vitro EGF, demonstrating EGFR activation in this tissue. IHC revealed EGF and EGFR expression in epithelial and interstitial cells and in luminal sperm along rat epididymis. pEGFR staining was observed in the same cell types expressing EGFR, demonstrating its constitutive activation. Bilateral EDL altered EGF and EGFR expression (mRNA and protein) only in IS and CP, although pEGFR immunodistribution was not changed. EGFR positive IF was observed in sperm from T (acrosome), IS and CP (acrosome and flagellum), CO (entire sperm) and CD (acrosome), whereas EGF was only immunodetected in sperm from T (acrosome).
Conclusions: The data show a segment specific pattern of EGF and EGFR expression along rat epididymis and indicate constitutive expression of pEGFR, suggesting that EGFR in epididymal cells is submitted to autocrine and/or paracrine activation. The dynamics of EGFR immunolocalization in the maturing sperm may also suggest an important role for this receptor in events leading to sperm maturation and, consequently, to male fertility.
Support: FAPESP, CAPES, CNPq, Fogarty International Center.

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PREPUBERTAL ANTIANDROGEN EXPOSURE: IMPAIRMENT OF THE EPIDIDYMAL FUNCTION IN ADULT RATS

Juliana Perobelli, BS[2], Carla Fernandez, MS[2], Fabíola Toledo, BS[2], Gary Klinefelter, PhD[3] and Wilma Kempinas, PhD[1] (Presented By: Wilma Kempinas)
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In recent years great attention has been given to chemical agents called endocrine disruptors. Previous works have shown the impact of exposure to antiandrogen chemicals in utero and during adult life on the rat epididymis. However, few studies have focused on possible consequences to the epididymis in rats exposed to antiandrogen during prepuberty, which is the moment this organ presents important changes that result in the regional differentiation of the duct, with particular morphology, function and gene expression. The present work aims to evaluate the impact of antiandrogen exposure, during prepuberty, on the fertile capacity of epididymal sperm of adult rats. Male Wistar rats, 21 days old, were randomly allocated into 2 experimental groups: treated (T, n = 9) that received, by oral route, daily doses of flutamide (Sigma Aldrich – 25 mg/Kg), and control (C, n = 10), that received the vehicle (corn oil). The treatment occurred from postnatal day (PND) 21 to 44. Sperm quality was evaluated using a fixed number of sperm collected from the proximal cauda epididymidis and artificially inseminated directly into the uterus of synchronized adult female rats. Fertility potential (FP) was assessed on gestation day 20. On PND 75 the animals were killed and had their body weight and reproductive organ weights recorded, and the right epididymal cauda used for the artificial insemination. Statistical analyses were performed using Student's "t" test and Mann-Whitney test (*$p<0.05$), and results are expressed as mean ± SEM. There was a significant delay in the onset of preputial separation in the T group. The body weights were similar between groups, but there was a significant reduction ($p<0.05$) in the vas deferens and seminal vesicle weights in the T group. The females that were inseminated with sperm from the T rats showed a lower FP (78.81 ± 6.39*) than the C group (94.68 ± 2.65) . There was also an increase of pre-implantation loss in the T group (21.19 ± 6.39*) compared to the C group (5.32 ± 2.65). As consequence, there were fewer implants (T = 9.56 ± 0.80*; C = 12.10 ± 0.62) in the uterus of females inseminated by T rats. These results show a diminished sperm quality in adult rats that had been exposed to antiandrogen during prepuberty, and suggest that androgens play a pivotal role in epididymal development. The morphology of the epididymis and the sperm membrane and epididymal tissue proteomes of these rats are under investigation.
Funding: CNPq, FAPESP.

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GLYCOMIC ANALYSIS OF HUMAN SEMINAL PLASMA REVEALS THE PRESENCE OF IMMUNOMODULATORY CARBOHYDRATE FUNCTIONAL GROUPS

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[1]Division of Molecular Biosciences, Faculty of Natural Sciences, Imperial College London, UK; [2]Department of Obstetrics, Gynecology and Women's Health, School of Medicine, University of Missouri, Columbia; [4]M-SCAN Ltd, Wokingham, UK

Introduction and Objectives: There is abundant evidence that human seminal plasma (SP) components are immunosuppressive *in vitro*, presumably limiting destruction of sperm in the male and female reproductive tracts. A recent study confirms that human sperm, which lack major histocompatibility class I molecules, display carbohydrate sequences known as bisecting type N-glycans (Pang et al, 2007. J Biol Chem 282(50):36593), associated with the suppression of natural killer cell-mediated responses. These gametes also express terminal Lewis^x (Le^x) and Lewis^y (Le^y) sequences implicated in suppression of the adaptive immune response. The current study was undertaken to determine if N- and O-glycans on SP glycoproteins also express these immunoregulatory sequences.
Methods: Both conventional and ultrasensitive mass spectrometric (MS) methods were employed. After purification of glycan, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS and tandem MS (MS/MS) data were acquired. The processed spectra were subjected to manual assignment and annotation. Peak picking was done manually, and proposed assignments for the selected peaks were based on molecular mass composition of the ^{12}C isotope together with knowledge of the biosynthetic pathways. The proposed structures were then confirmed by data obtained from MS/MS experiment.
Results: Three major families of N-glycans were detected: (i) high mannose glycans $Man_{5\text{-}8}GlcNAc_2$; (ii) bi-, tri- and tetraantennary core-fucosylated complex type N-glycans with antennae terminated with Le^x and/or Le^y sequences; (iii) bi-, tri- and tetraantennary core-fucosylated complex type N-glycans whose antennae are capped with sialic acid. Core 1 and Core 2 O-glycans are also fucosylated or sialylated or a combination of both.
Conclusions: The same high mannose and polyfucosylated N-glycans associated with sperm are also present in SP, though bisecting type N-glycans are far less abundant than in sperm. SP glycoproteins also present substantial sialylated N-glycans that act as ligands for siglecs and galectins. In summary, the immune response in the male and female reproductive systems may be regulated by the addition of carbohydrate functional groups to both sperm and SP glycoproteins, protecting sperm,

which bear antigens foreign in both systems. Such carbohydrate sequences could also be one of the central factors that establishes immune privilege in the urogenital tract organs in the human male.

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DOES THE DECREASE IN SPERM P34H CORRELATE WITH THE TIME ELAPSED BETWEEN VASECTOMY AND VASOVASOVASTOMY

Luc Boudreau, Michel-Hugues Lebel, MD, Annie Imbeault, MD, Christine Légaré, MSc, Robert Sullivan, PhD and Michel Thabet, MD, FRCSC
(Presented By: Luc Boudreau)
Université Laval

Introduction: During epididymal transit, spermatozoa acquire new surface proteins. P34H being one of them, it is implicated in sperm-zona pellucida binding. Absence of this surface protein is associated with inability of sperm to interact with the oocyte. We have previously shown that P34H was present in all fertile men and approximately in 50 % of idiopathic infertile men. We also showed that P34H remains at the same level in different semen from a given individual.
Purpose: Vasovasostomy has a high surgical success rate but fertility is not recovered in a significant proportion of cases. Knowing that vasectomy can cause epididymal damages, we have quantified P34H in vasovasostomized men with regards to the time elapsed between vasectomy and surgical reversal.
Methods: We retrospectively analyzed 289 semens of patients between 1997 and 2006 who underwent microsurgical two-layer microsurgical vasovasostomy performed by a single surgeon. P34H evaluated by western blot analysis was performed on a constant number of spermatozoa was available for 207 patients. Of these, 9 patients were excluded due to protein degradation on western blots.
Results: P34H decrease in a steady fashion for the first 9 years of interval between vasectomy and vasovasostomy. In fact, 94 % of patients that were under vasectomy for less than 3 years had a positive P34H. The decrease of P34H continues to reach 72 % of positivity between 7 to 9 years after vasectomy. Afterward, P34H was positive in 93% and 100% of vasovasostomized men after 10 to 12 years and 13 to 15 years under vasectomy respectively.
Conclusion: Our results show that a decrease of P34H is associated with the time elapsed between vasectomy and its surgical reversal at least for the first 9 years. After this period, a normal value of P34H characterises a high proportion of vasovasostomized men suggesting an adaptive mechanism within the epididymis. For patients with less then 10 years under vasectomy, the time elapsed between vasectomy and its reversal can be a part of the prognostic factors of fertility recovery in men.
Supported by CIHR grants to RS

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THE ANTI-MICROBIAL BETA-DEFENSIN 23 IS EXPRESSED IN RODENT EPIDIDYMIS AND BINDS TO SPERMATOZOA DURING THEIR EPIDIDYMAL TRANSIT

Justin Miles, Laura Piehl, Kathy Bowlin and Antoine Makhlouf, MD, PhD
(Presented By: Antoine Makhlouf)
University of Minnesota

Introduction: Beta-defensins are antimicrobial proteins that are preferentially expressed in the male excurrent reproductive system. Many novel defensins have been discovered via computerized genomic analysis, but their function and protein expression pattern remain largely unknown. Proteomic analysis of human ejaculate suggested that DEFB129 is the most abundant defensin in humans.
Aim: Characterize the expression of Defb23, the rodent ortholog of human DEFB129, in rats.
Methods: A polyclonal antibody was developed against a peptide fragment of Defb23 and its specificity confirmed by immunoblotting using recombinant Defb23 and peptide competition. Immunocytochemistry and immunoblot analysis was then used to characterize expression of Defb23 in rat testis and epididymis.
Results: Defb23 was expressed in the epithelium of rat distal caput and cauda epididymis, as well as the seminiferous epithelium of the testis. Immunocytochemistry of sperm cells revealed it to be present in a distinct pattern in the head region, with an increase in signal in cauda sperm. Immuoblot analysis confirmed its presence in epididymal cells with as well as epididymal sperm at a MW of ~ 18 KD. Preliminary studies reveal that recombinant Defb23 does not possess potent anti-microbial activity.
Conclusions: this is the first report of Defb23 expression at the protein level in the rodent epididymis. Defb23 appears to bind to sperm heads during their transit in the epididymis.

Monday, April 6, 2009
11:00 a.m. – 12:30 p.m.

Poster Session II
Location: Grand Ballroom, 2nd Floor

MALE SEXUAL FUNCTION

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DECREASED AKT SIGNALING IN THE CORPORAL TISSUE OF HYPERCHOELSTEROLEMIC APOLIPOPROTEIN E KNOCKOUT MICE

Donghua Xie, MD, PhD, Lijing Jia, MD, PhD, Feihua Wu, BA and Craig Donatucci, MD (Presented By: Donghua Xie)
Duke University Medical Center

Introduction and Objective: Apolipoprotein E knockout (ApoE-/-) mouse is a classic atherosclerosis animal model with spontaneous hypercholesterolemia. Hypercholesterolemia is the major risk factor for the development of erectile dysfunction (ED). Akt plays a critical role in controlling survival and apoptosis. GSK-3a/â and p70 S6 kinase are important downstream molecules of Akt signaling. We sought to determine whether there are differences in Akt signaling in the corporal tissue of ApoE-/- mice.
Materials and Methods: Ten adult wild-type C57BL6 (Bl-6) (Group 1, n=10)) and 10 age matched ApoE-/- mice were used for this study (Group 2, n=10). Corporal tissues were harvested and studied for level of cyclic guanosine monophosphate (cGMP) by enzyme immunoassay assay (ELISA); Levels of Akt, phosphorylated Akt, phosphorylated GSK-3a/â and phosphorylated p70 S6 kinase were assessed by western blot analysis. Results: Akt, phosphorylated Akt, phosphorylated GSK-3a/â, phosphorylated p70 S6 kinase, and cGMP were significantly decreased in the corporal tissue of ApoE-/- mice compared to that of wild-type Bl-6 mice. Conclusions: ApoE-/- mice determine lower basic level of Akt. Impaired Akt signaling may lead to decreased cGMP production and this pathway may be exploited for the treatment of hypercholesterolemia induced ED.

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DECREASED AKT ACTIVATION AND INCREASED PTEN EXPRESSION IN THE CORPORAL TISSUE OF MICE WITH ALLOXAN INDUCED DIABETES

Donghua Xie, MD, PhD, Lijing Jia, MD, PhD, Feihua Wu, BA and Craig Donatucci, MD (Presented By: Donghua Xie)
Duke University Medical Center

Introduction and Objective: Akt is a protein kinase that plays a critical role in controlling survival and apoptosis. It is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase. PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a major negative regulator of the PI3K/Akt signaling pathway. We sought to investigate the changes in PTEN/Akt signaling in type 1 diabetes mellitus (DM) induced erectile dysfunction (ED) in mice. Material/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 level of cyclic guanosine monophosphate (cGMP) by enzyme immunoassay assay (ELISA); Levels of Akt, phosphorylated Akt, and PTEN were assessed by western blot analysis. Results: Akt phosphorylation and cGMP and were significantly decreased while PTEN was significantly increased in the corporal tissue of diabetic mice. Conclusions: The accompanying decrease in cGMP maybe a result of PTEN/Akt dysregulation and this may have an effect on erectile function.

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MATHEMATICAL VALIDATION OF DUPLEX DOPPLER ULTRASOUND DATA TO PREDICT PENILE HEMODYNAMICS IN MEN WITH ERECTILE DYSFUNCTION

Suresh Sikka, PhD, GurdialArora, PhD[1], Tumulesh Solanky, PhD[2] and Wayne J.G. Hellstrom, MD[3] (Presented By: Suresh Sikka)
[1]Xavier University; [2]University of New Orleans; [3]Tulane University

Objective: Penile duplex Doppler ultrasound (PDDU) data was used to construct a mathematical model to predict impact of comorbidities on penile hemodynamics in men with erectile dysfunction (ED).
Methods:
Using a 10 mHz ultrasound probe, the diameter of each cavemosal artery, peak systolic blood flow velocity (PSV), end-diastolic velocity (EDV), and resistance index (RI) were evaluated at various time intervals before and after a single intracorporal injection of 7 to 20 micrograms of prostaglandin E1 (PGE1). A multivariate regression model was constructed using such PDDU data from 500 ED patients to correlate various predictors and comorbidities.
Results: For assessing the accuracy of the prediction model, the data was divided into two parts: the first part had 400 observations that were used to fit the statistical prediction model, while the remaining 100 observations were used to test the accuracy of such model. In this intuitive model, observed blood flows at different time points were plotted versus predicted blood flow values. The prediction model was able to accurately classify 66.7% of the observed values. The R-Square for the regression model was 0.9037 for PSV and 0.6846 for EDV. The principal component analysis did not show any significant effect on predictability of these velocities. Diabetic men with ED demonstrated lower PSV compared to non-diabetic patients at 15 minutes post injection. These differences in PSV were more pronounced at 15 and 20 micrograms PGE1 than at lower doses. No significant differences in RI and EDV were observed in the two groups.
Conclusions: Both predictive and observed Doppler data, contrary to common belief, show that diabetic men with ED are more likely to demonstrate arterial insufficiency rather than veno-occlusive dysfunction; and that at least 15 micrograms PGE1 should be used for the intracorporal injection especially in suspected venous-leakage ED patients.

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ENHANCED ALPHA 1-ADRENOCEPTOR AND P2X-PURINOCEPTOR MEDIATED RESPONSIVENESS IN VAS DEFERENS OF NITRIC OXIDE-DEFICIENT HYPERTENSIVE RATS: PARTIAL PROTECTION BY SILDENAFIL

Serap Gur, PhD[1], Suresh Sikka, PhD[2] and Wayne Hellstrom, MD[2] (Presented By: Serap Gur)
[1]Tulane Urology; [2]Tulane University

Introduction: Hypertension (HT) is a major risk factor for erectile dysfunction (ED). N(G)-nitro-L-arginine methyl ester (L-NAME), a NO synthase (NOS) inhibitor, is used for induction of NO-deficient HT. Our aim was to investigate the effects of L-NAME-induced HT in the rat vas deferens and to determine if sildenafil provides and protective or regenerative effects.
Materials and methods: Thirty six rats were divided as: (1) control, (2) L-NAME-HT, (3) sildenafil-treated L-NAME-HT. Group 2 was treated with L-NAME (40 mg/rat/day) in drinking water for four weeks. Group 3 received sildenafil (5 mg/ day/rat, by oral gavage) concomitantly with L-NAME. Strips of vas deferens (VD) were suspended in an organ bath and subjected to electrical stimulation to establish frequency-response curves, P2X1 agonist á,â-meATP, and alpha-1 agonist phenylephrine. To determine the involvement of the P2X1 receptor on EFS induced contractions, these experiments were repeated in the presence of pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, P2X receptor antagonist).
Results: Contraction by EFS (C; 0.61 ±0,10, L-NAME-HT; 1.05 ± 0,042, p<0.05 and SIL-NAME-HT group; 0.42 ±0.068 g/ g tissue), P2X1 agonist á,â-meATP (C; 0.394 ±0.049, L-NAME-HT; 1.06 ± 0,013 (p<0.05) and SIL-NAME-HT group; 1.036 ±0.014 g/g tissue), and á-1 agonist and phenylephrine (C; 0.37 ±0.058, L-NAME HT 0.77 ± 0.056 p<0,01; SIL-NAME-HT: 0.85 ±0.102 p<0.01) in vitro was significantly enhanced in the VD of L-NAME group (#2). Sildenafil treatment in the L-NAME group (#3) significantly improved contractile response to EFS of VD. In the presence of the P2X1 antagonist PPADS, the enhanced contractile response to EFS in isolated VD from L-NAME rats was resumed. Potassium chloride (KCl) induced contractile responses were not changed (C; 1.03 ±0.017; L-NAME-HT-0.98 ±0.096; SIL-HT- 1.07 ± 0.02 g/ g tissue).
Conclusions: Upregulation of NO-sGS-cGMP pathway by sildenafil may have a suppressive effect on the enhanced release of ATP from NANC nerves, but not the purinergic or adrenergic post-receptor signaling system in VD. Thereby, P2X1 receptor antagonism related therapeutic approaches may be beneficial in treating ejaculatory dysfunction in hypertensive men.

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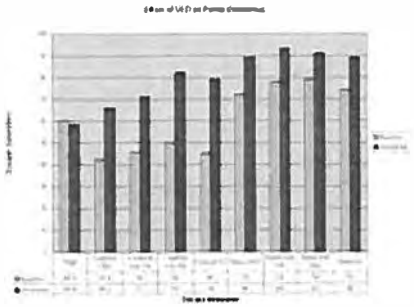
ACUTE CHANGES IN PENILE OXIMETRY AFTER SINGLE UTILIZATION OF THE VACUUM ERECTION DEVICE (VED) COMPARED TO INTRAURETHRAL ALPROSTATDIL (IUA) AND INTRCAVERNOSAL INJECTIONS (ICI) IN PATIENTS FOLLOWING RADICAL PROSTATECTOMY (RP)

Artrit Bytyci, BS, Brianne Goodwin, BS, Joseph Alukal, MD and Andrew McCullough, MD (Presented By: Artrit Bytyci)
New York University

Introduction: Many patients recovering from RP experience erectile dysfunction (ED) and left unmanaged the ED can lead permanent impairment of erectile function. Studies have shown that intraurethral alprostadil (IUA) and intracavemosal injections (ICI) provide oxygenated blood to the corpora in these patients, which may prevent or reduce corporal fibrosis. The level of corporal oxygenation achieved during vacuum therapy has never been demonstrated. This trial assessed the immediate effects of VED application without the constriction band on penile oximetry in RP patients. Results were then compared to similar cohorts of men following single IUA and ICI administration.
Methods: Twenty men between 2 and 24 months following RP were enrolled. Under supervision, each man cycled the VED to full erection 10 consecutive times over a period of approximately 5 minutes. Penile oximetries were immediately measured using the Vioptix Odissey oximeter at five marked sites: the right thigh, right corpora, glans penis, left corpora, and left thigh. At least 5 measurements were made at each site. These results were compared with those obtained in a prior cohort of men after the application of IUA 125mcg (n=21), IUA 250mcg (n=18), and ICI of trimix (n=54)).
Results: Mean age and time from surgery was 58.2 years and 13.1 months respectively, and the average SHIM score was 7. Right and left oximetry measurements were averaged. All techniques significantly (p<0.001) increased corporal and glanular oximetry relative to baseline. No change was observed in thigh oximetry. Upon comparison with separate, patients cohorts, the VED improved corporal oxygenation comparably to IUA 125 but less than IUA 250 and ICI.

Conclusion: This is the first study demonstrating that a single, brief application of the VED without a constriction ring results in significant improvement in both corporal and glanular oxygen saturation. While IUA 250ug and ICI appear to have improved corporal oximetry to a greater degree in separate, unmatched cohorts, the VED has significant benefits for patients both with regard to cost and invasiveness. This study supports incorporation of the VED in post-prostatectomy penile rehabilitation protocols.



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THE CLINICAL ASSOCIATION BETWEEN FREE ANDROGEN INDEX AND ERECTILE DYSFUNCTION IN MEN FROM THE MIDDLE EAST

Ali Thwaini[4], A Hameed[4], M Z Aslam[4], I Shergill[1], Raed Ahmed[2], Gada Yahia[2], Donald Morgan[3] and Frank Chinegwundoh[4] (Presented By: Ali Thwaini)
[1]Haroldwood Hospital UK; [2]Tawam Hospital, UAE; [3]Sheikh Khalifa Medical Centre, Abu Dhabi; [4]Bart's and the London Hospitals, UK

Objectives: To assess the relation of sex hormone levels in men, as measured by Free Androgen Index (FAI), with severity of erectile dysfunction (ED) and with their response to treatment.
Methods: We retrospectively reviewed the medical records of men who consecutively attended the Urology Clinic with the complaint of ED between March 2000 and October 2003. The Sexual Health Inventory for Men (SHIM) score was used as the main outcome measure in this study, and its variation was tested by certain variables using the Epi Info software ®.
Results: A total of 150 men were studied. The majority of patients (93%) had FAI in the normal range levels, and had shown no relation to the SHIM score even after adjustment for other factors (P = 0.16). However, FAI was highly related to patients' response to treatment (P =0.001), with the higher the level the higher was the proportion of patients responded well to treatment. Age of the patient was the only factor influencing the SHIM score they could attain, as shown by the linear regression analyses (P = 0.004).
Conclusion: The FAI level is not related to the severity of ED. Its role however, is confined to the way patients are going to respond to medical treatment of ED. Further studies are therefore needed to assess the effectiveness of using this parameter as a reliable test of bioactive testosterone for men with ED.

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PARTIAL PENILE SENSORY AXON RESECTION (PPSAR) FOR MANAGEMENT OF EJACULATION PRAECOX

Aref Elseweifi, consultant of urology (Presented By: Aref Elseweifi)
Private institute

Ejaculatory latency is biologically and psychologically controlled. Psychosexual counseling and behavioral treatment can be ineffective. Selective serotonin reuptake inhibitors, lignocaine, prilocaine, and phosphodiasterase inhibitors are of limited value. PPSAR is done through bilateral resection of the nerve twigs of the dorsal nerve of the penis, fanning laterally and ventrally to innervate the glans at the subglandular level leading to partial hyposensitivity of the glans penis, which increases ejaculatory latency and hence improves premature ejaculation (PE). Twenty-three patients with a mean age of 34.5 years were treated during the last 28 months. The pre-operative reported ejaculatory latency was 2 seconds to 4 minutes. Two patients (8.7%) showed no improvement. Diabetes mellitus and arteriogenic impotence may have been the cause of failure in both patients. Twenty-one patients (91.3%) reported on prolonged latency up to 7-20 minutes. Three of them (13%) reached satisfactory success using postoperative local application of Xylocain 1% ointment. Slight coldness of the glans was observed in one patient (4.3%) where some fibers of the dorsal nerve were resected while anaesthesia at the tip of the glans was observed in another (4.3%). All patients were free of major postoperative complications. No correlation was found between the amount of the resection and the postoperative outcome. The results were evaluated through a standard questionnaire. Partial penile neurectomy is a promising operative technique for the treatment of PE.

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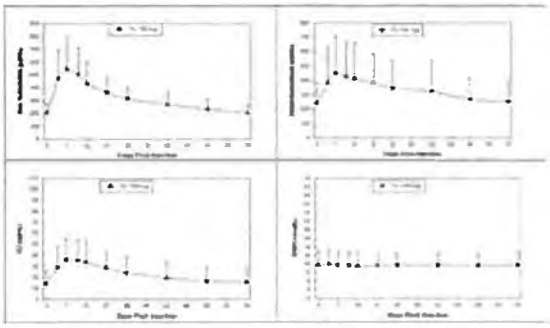
IMPACT OF LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION ON SEX HORMONES IN HYPOGONADAL MEN

Wayne Hellstrom, MD[1], Leonard Marks, MD[2], Ronald Swerdloff, MD[2], Christina Wang, MD[2], Joel Kaufman, MD, Evan Goldfischer, MD[3], Martin Miner, MD[4] and Ridwan Shabsigh, MD[5] (Presented By: Joel Kaufman)
[1]Tulane University School of Medicine; [2]David Geffen School of Medicine at UCLA, LA, CA; [3]Vassar Brothers Medical Center, Poughkeepsie, NY; [4]The Warren Alpert Medical School of Brown University, Providence, RI; [5]Maimonides Medical Center and Columbia University, New York, NY

Introduction and Objectives: Analysis conducted to evaluate the effects of long-acting testosterone undecanoate (TU) 750 mg intramuscular (IM) injection every 10 wks on sex hormones in hypogonadal men.
Methods: Hypogonadal men were treated with TU 750 mg IM (n=130) at baseline, wks 4, 14, and 24. DHT, E2, and SHBG were sampled at days 0, 4, 7, 11, 14, 21, 28, 42, 56, and 70. Serum hormone concentrations and their ratio to serum T concentrations were assessed.
Results Obtained: Patient's mean age was 54 yrs and mean body mass index was 31.5 kg/m2. Mean concentration of DHT and E2 tracked concentrations of T, while mean SHBG remained unchanged. Concentrations of these hormones and their ratios to T remained in the normal range throughout the dosing interval. (Figure).
Conclusions: This 24-week study demonstrated that treatment with TU 750 mg IM every 10 wks after initial loading dose at 4 weeks did not adversely affect sex hormones in men with hypogonadism. This study was funded by Indevus Pharmaceuticals.
Figure: Ratio of Sex Hormones to Testosterone Following the 3rd Injection of TU 750 mg



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IMPROVED MALE SEXUAL FUNCTION WITH LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION

Wayne Hellstrom, MD, Leonard Marks, MD[1], Ronald Swerdloff, MD[1], Christina Wang, MD[1], Joel Kaufman, MD[2], Evan Goldfischer, MD[3], Martin Miner, MD[4] and Ridwan Shabsigh, MD[5] (Presented By: Wayne Hellstrom)
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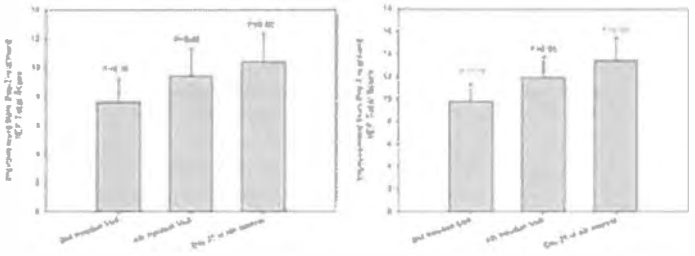
Introduction and Objectives: Analysis to evaluate the effect of long-acting testosterone undecanoate (TU) 750 and 1000 mg intramuscular (IM) injection on sexual function of hypogonadal men.
Methods: Hypogonadal men randomized in US multicenter study to receive TU 750 (n=102) or 1000 mg (n=97) IM every 12 weeks for 48 weeks, for a total of 5 injections. The International Index of Erectile Function (IIEF) was collected at baseline, 2nd, and 4th injection, (week 0, 12, 36, respectively) to assess the effects of TU on sexual functioning.
Results Obtained: Treatment with TU 750 and 1000 mg IM resulted in significant improvements for all IIEF domains, including erectile function, satisfaction with intercourse, orgasmic function, sexual desire, and overall sexual satisfaction. Improvement in IIEF domains were seen as early as 21 days following the first injection of TU and continued for study duration (Figure).
Conclusions: This 48-week study demonstrated that treatment of hypogonadal men with TU 750 and 1000 mg IM resulted in significant improvements in erectile function and other markers of sexual satisfaction. This study was funded by Indevus Pharmaceuticals.
Figure – Effects ofTU 750 and 1000 mg IM on IIEF Scores



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EFFECTS OF LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION ON PROSTATE HEALTH OUTCOMES IN HYPOGONADAL MEN

Wayne Hellstrom, MD[1], Leonard Marks, MD[2], Ronald Swerdloff, MD[2], Christina Wang, MD[2], Joel Kaufman, MD, Evan Goldfischer, MD[3], Martin Miner, MD[4] and Ridwan Shabsigh, MD[5] (Presented By: Joel Kaufman)
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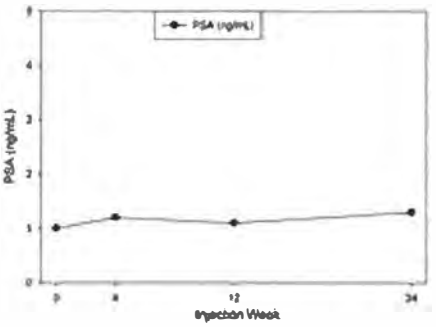
Introduction and Objectives: Analysis conducted to evaluate effects of long-acting testosterone undecanoate (TU) 750 mg intramuscular (IM) injection every 10 weeks on prostate health of hypogonadal men.
Methods: Hypogonadal men were treated with TU 750 mg (n=130) at baseline, weeks 4, 14, and 24. Prostate specific antigen (PSA) and digital rectal examinations (DRE) were performed pretreatment and at each injection; PSA was obtained prior to DRE.
Results Obtained: Patients mean age: 54 years, mean body mass index: 31.5 kg/m2. Mean PSA increased <0.3 ng/mL over this 24-week study; 2.3% (3/129) of patients had a new-onset postbaseline PSA >4 ng/mL (Figure); 11 (8.5%) had an abnormal DRE at any time post-1st injection. Adverse events associated with prostate health included prostatitis [n=3 (2.3%)] and increased PSA [n=2 (1.5%)]. No patient was diagnosed with prostate cancer during the study.
Conclusions: This 24-week study showed that treatment with TU 750 mg IM every 10 wks resulted in no clinically meaningful changes in PSA or DRE findings. There was no evidence of unexpected prostate health outcomes. This study was funded by Indevus Pharmaceuticals.
Figure – Mean PSA through 24 weeks of treatment.



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TIME DEPENDENT CHANGES IN PENILE OXIMETRY AFTER SINGLE APPLICATION OF VACUUM ERECTION DEVICE (VED) IN MEN AFTER RADICAL PROSTATECTOMY (RP)

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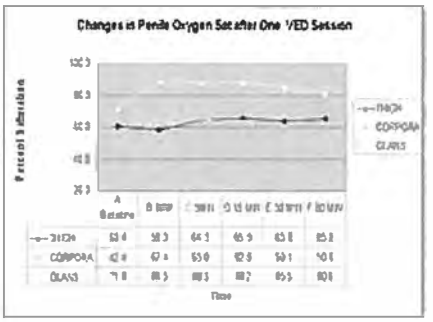
New York University

Introduction: The VED has been incorporated in penile rehabilitation after radical prostatectomy. The effect of the VED without a constriction ring has never before been evaluated. We examined the time dependent changes on penile oxygen saturation after a brief application of the VED without the constriction device.
Methods: Twenty men between 2-24 months from RP were enrolled. None had used erectogenic aides for at least one week. Under supervision, each man cycled the VED to full erection 10 consecutive times over a period of approximately 5 minutes. Penile oximetries were measured using the Vioptix Odissey oximeter; at five marked sites, the right thigh, right corpora, glans penis, left corpora, and left thigh. At least 5 measurements were made at each site. The measurements were repeated immediately, 5, 15, 30 and 60 minutes after use of the VED.
Results: Mean age and time from surgery was 58 and 12.6 months respectively. Average SHIM score was 7. Right and left oximetry measurements were averaged. Corporal and glanular oximetries increased immediately and remained significantly elevated for 30 minutes. Glanular oximetries remained elevated at 60 minutes. No change was observed in thigh oximetry. In 7 men with preoperative measurements, baseline corporal levels were significantly lower than preoperative levels ($p<0.02$)
Conclusion: The brief use of VED without constriction ring results in significant improvement in both corporal and glanular oxygen saturation for at least 30 minutes. The study supports incorporating the VED in penile rehabilitation post RP to increase penile oxygenation. Repeated or longer daily applications may be of additional benefit.



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IMPROVED DEPRESSION-RELATED OUTCOMES IN HYPOGONADAL MEN TREATED WITH LONG-ACTING TESTOSTERONE UNDECANOATE INTRAMUSCULAR INJECTION

Wayne Hellstrom, MD, Leonard Marks, MD[1], Ronald Swerdloff, MD[1], Christina Wang, MD[1], Joel Kaufman, MD[2], Evan Goldfischer, MD[3], Martin Miner, MD[4] and Ridwan Shabsigh, MD[5] (Presented By: Wayne Hellstrom)
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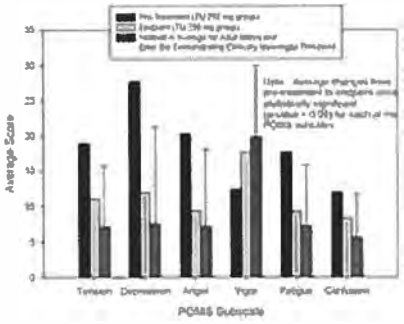
Introduction and Objectives: Analysis conducted to evaluate effects of long-acting testosterone undecanoate (TU) 750 mg intramuscular (IM) injection on depression-related outcomes in hypogonadal men.
Methods: Hypogonadal men were randomized to receive TU 750 mg (n=120) IM every 12 weeks for 48 weeks. The Profile of Mood State (POMS) was collected at each injection visit through the 5th injection and 21 days following the 4th injection.
Results Obtained: Treatment with TU 750 mg IM, in a subset of clinically depressed patients (n=19), resulted in statistically significant (P<.05) changes in Total Mood Disturbance Score and all POMS subscales (Figure).
Conclusions: Treatment with TU 750 mg IM every 12 weeks for 48 weeks provided statistical and clinical improvements in hypogonadal men with depression. This study was funded by Indevus Pharmaceuticals.
Figure. 750 mg TU results in clinically meaningful POMS subscale scores in patients clinically depressed at pretreatment. Lower scores on the POMS represent improved mood state (less tension-anxiety, depression-dejection, etc.); negative correlations indicate improvement on the POMS as T concentrations increase.



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PENILE AUGMENTATION SURGERY USING AUTOGRAFT, ALLOGRAFT OR XENOGRAFT

Joon Yong Kim, MD[1], Byung Moo Philip Kim, MR[1], Si Jin Paul Kim Kim, MR[1], Hee Kyung Kim, professor[2] and Beom Joon Kim, professor[3] (Presented By: Joon Yong Kim)
[1]Philip and Paul Medical Institute; [2]SoonChunHyang University College of Medicine; [3]Dermatology, Chung-Ang University

Introduction: Up to present autologous dermal-fat , fat graft and allograft have been used and xenograft is used these days in surgery. This study is to report on penile augmentation surgery using autograft, allograft, and xenograft.
Material and Method: We performed complex phalloplasty that enables simultaneous surgeries for glans augmentation, penile lengthening, and girth enhancement. Autologous dermal-fat, acellular allogenic dermal graft or heterogenic type I collagen. The thickness of the augmented graft was about 4-6mm. The material was designed according to the size of the penis and then grafted. In the penile skin of the dorsal area, 1-2 cm from the subglans or circumcised scar was minimally incised at a length of 3 to 5 cm in a transverse direction Enhancement tissue was placed between the dartos and Buck's fascia and fixed to the Buck's fascia in the penis. Histologic examination was conducted for people that took penile augmentation surgery at least one year earlier.
Result: The surgery was conducted on 720 patients from January 2005 to December 2007. The thickness of the augmented graft was about 3-6mm in a few examined cases by ultrasonography. The grafted tissues were well combined with the host penile stromal tissue with new vessel proliferation revealed and did not show any abnormal histologic changes in a few histologically examined cases.
As for complications, in case of graft failure, the graft was removed partially or entirely. Some necrosis cases occurred but most of them were treated through conservative treatment. Most complications were transient and patients recovered through conservative treatment.
Conclusions: Penile augmentation surgery using the above described grafted tissues showed positive results in terms of safety, effectiveness and histologic findings. This study reports that those materials may be considered as safe materials for penile augmentation surgery.

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PARTIAL PENILE AUGMENTATION SURGERY WITH MULTIPLE PUNTURE SLING METHOD

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[1]Philip and Paul Medical Institute; [2]Chung-Ang University, Dept. of Dermatology

Purpose: Penile augmentation surgery has been conducted in two ways so far. A general method is to insert graft through an incision at the distal area of penis or prepubic area. Another penile augmentation surgery is to inject graft or material not through the method of incision. Meanwhile, due to the wrong augmentation surgery that was used in the past, some cases show unnatural shape wherein it seems that the glans has separated from the shaft. We applied a multiple puncture method using hetero type I collagen or autologous dermis for those who wanted to correct such an unnatural appearance or those who wanted partial penile augmentation.
Subjects and Methods: For the graft, a hetero type I collagen or autologous dermal strip of 5-10 mm in width, 5-7mm in thickness, and 4-6 cm in length was provided as required for augmentation. The procedure is to puncture the ventrolateral side of the penis that is to be augmented or corrected in a 5 o'clock direction with scissors having sharp ends and then make a space to dorsal area of penis for the strip with a thin dissector and then penetration through the outer skin on the opposite side in a 7 o'clock direction. Hold the prepared strip with by seizing forceps and drag to the 5 o'clock direction to balance it. If required, the perforated part may be sutured with one stitch. If additional augmentation is required to other part, repeat the procedure on the part as above.
Results: The clinical results of the surgeries conducted from August 2006 to May 2008 were as follows. The subjects were 24 in total and the number of strips used in one surgery was two on average. The surgery time was 25 minutes on average. The adverse effects reported were one case of infection. No special visit to a doctor was required after the surgery and the patients were able to return to daily life immediately after the surgery.
Conclusion: Partial penile augmentation surgery that applied minimal invasive multiple puncture method with hetero type collagen strips did not require incision and had fast recovery time. There was little adverse effect and the surgery method was simple. Therefore, it can be positively considered to apply for those who want partial augmentation surgery and those who want to correct any unnatural appearance due to the thickness gap between the augmented part and non-augmented part after augmentation surgery using the preexisting method.

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LIQUID CHROMATOGRAPHY-MASS SPECTROMETRIC ASSAY OF TESTOSTERONE AND CORTISOL IN SALIVARY FOR DIAGNOSING MALE ANDROPAUSE

Eitetsu Koh, PhD, MD, Futoshi Matsui, PhD, MD, Kazuhiro Sugimoto, MD, Hosu Sin, MS, Yuji Maeda, PhD, MD and Mikio Namiki, PhD, MD (Presented By: Eitetsu Koh)
Kanazawa University Graduate School of Medical Science

Introduction: It is well known that andropause in males can cause a variety of symptoms, and the differential diagnosis is relatively difficult, including psychological disorders, stress, and mood disturbances. The level of serum cortisol can be measured to reflect a patient's level of stress. Salivary hormones facilitate the evaluation of physiological hormonal actions based on free hormone assay.
Methods: For the simultaneous measurement of testosterone and cortisol levels in saliva, we developed a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.
Results: On LC-MS/MS, m/z 289.2/97.3 for testosterone and m/z 363.3/327 for cortisol were regarded as the quantitative ion. Concerning accuracy and precision, the lower limit of quantity of salivary testosterone and cortisol were established as 5 and 10 pg, respectively. The measurement of testosterone and cortisol in saliva is stable for 2 days, 14 days, and 28 days at room temperature, refrigeration and freezing, respectively. Freezing and thawing and stimulation of salivation with gum chewing does not alter the measured values of testosterone and cortisol. Total, bioavailable, and free serum testosterone showed slight diurnal changes, but total and bioavailable serum cortisol showed marked diurnal changes. Salivary testosterone levels negatively correlate with age, regardless of the time of saliva collection ($r=0.64$, $p<0.05$). However, there is no relationship between salivary cortisol and age ($r=0033$, $p>0.05$).
Discussion and Conclusion: LC-MS/MS allows rapid, simultaneous, sensitive, and accurate quantification of testosterone and cortisol in saliva for the diagnosis andropause or other hormone related disease

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INDUCTION OF SPERMATOGENESIS AND FERTILITY DURING GONADOTROPIN TREATMENT OF GONADOTROPIN DEFICIENT INFERTILE MEN: CLUES TO IMPROVING FERTILITY OUTCOME

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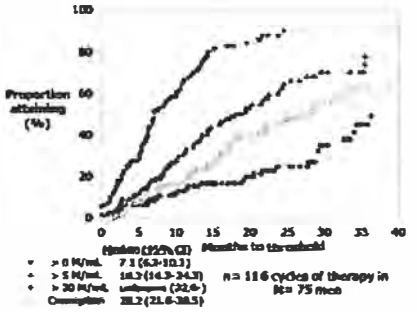
[1]University of Sydney; [2]University of Melbourne

Background: The induction of spermatogenesis and fertility with gonadotropin therapy in gonadotropin deficient men varies in rate and extent. Understanding the predictors of response would inform clinical practice, but requires multivariate analyses in sufficiently large clinical cohorts that are suitably detailed and frequently assessed. Previous studies of gonadotropin therapy in gonadotropin deficient men each included fewer than 30 men, and fewer used a sophisticated correlated time-to-event analysis to allow for multiple treatment cycles and subject drop outs.
Design, Setting and Participants: Seventy-five men, 72 desiring fertility, were treated at two academic Andrology centres for a total of 116 courses of therapy from 1981 until 2008.
Outcomes: Semen analysis and testicular examination every three months.
Results: Thirty-eight men became fathers, including 5 through assisted reproduction. The median time to achieve first sperm was 7.1 (95% CI 6.3 – 10.1) months and for conception was 28.2 (95% CI 21.6 – 38.5) months. The median sperm concentration at conception for unassisted pregnancies was 8.0 (95% CI 0.2 – 59.5) M/mL. Multivariate correlated time-to-event analyses show that larger testis volume, previous treatment with gonadotropins and no previous androgen use each independently predict faster induction of spermatogenesis and unassisted pregnancy.
Conclusion: Larger testis volume is a useful prognostic indicator of response. The association of slower responses following prior androgen therapy suggest that faster pregnancy rates might be achieved by substituting gonadotropin for androgen therapy for pubertal induction, although a prospective randomized trial will be required to prove this.



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SERUM INHIBIN B, BUT NOT FSH, LEVELS ARE SIGNIFICANTLY ALTERED IN OBESE MALES

Tyl Taylor, BS, MS[1], Micheal Glasner, MD[1] and William Roudebush, BS, MS, PhD[2] (Presented By: Tyl Taylor)
[1]Main Line Fertility; [2]Beckman Coulter, Inc.

Objective: In the human male, inhibin B is the principle gonadal feedback regulator of FSH secretion. Inhibin B is a glycoprotein hormone produced by the Sertoli cells of the testes and suppresses pituitary synthesis of FSH. Inhibin B may also have additional paracrine roles within the testes. Low serum inhibin B levels are indicative of poor or absent spermatogenesis. Body mass index (BMI) has been demonstrated to negatively impact sperm quality (i.e. chromatin integrity) and quantity (i.e. normal-motile counts) as well as reproductive endocrine levels (i.e. lower serum testosterone). Little to no information is available with regards to the relationship between inhibin-B and FSH levels and BMI. Therefore the study objective was to determine the relationship between inhibin B and FSH along with BMI.
Design: Comparison of BMI scores in male patients and serum FSH and inhibin B levels.
Methods: Patient height (H) and weight (W) were recorded day of serum collection. BMI was calculated for each patient as follows: (W*704.5)/(H2). Serum inhibin B levels were determined by enzyme-linked immunosorbent assay (ELIZA; Beckman Coulter, Inc., Chaska, M). Serum FSH levels were determined by data were analyzed by regression analysis and Student's t-test.
Results: A total of 122 serum levels were analyzed as described. The overall means (+SEM) for each parameter was as follows: BMI, 28.65 (+0.61) Kg/M2; FSH, 5.21 (+0.58) mIU/mL; and inhibin B, 8.96 (+1.19) pg/mL. Linear regression analysis ($R2=0.19$) revealed a significant ($P<0.05$) and negative relation [Inhibin B = 191-(2.9*BMI)] between BMI and serum inhibin B levels. Men presenting with a BMI >30 Kg/M2 (75.15 pg/mL) have significantly ($P<0.05$) lower inhibin B levels than men presenting with BMI's <30 Kg/M2 (119.85 pg/mL). There was no significant relationship between FSH and BMI.
Conclusion: An inverse relationship between BMI and serum inhibin-B was observed. Men whose BMI is greater than 25 have reduced serum inhibin-B, this being exacerbated when BMI is greater than 30. A male presenting with a high BMI is typically found to have abnormal serum inhibin-B, but not FSH, levels in addition to an abnormal semen analysis. BMI Additional studies are warranted to determine the impact BMI has upon male fertility.

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AN OPEN LABEL PHASE III STUDY OF FORTIGEL™ (TESTOSTERONE) 2% GEL IN HYPOGONADAL MALES

Adrian Dobs, MD[1], John McGettigan, MD[2], Mark Akerson, MD[3], Paul Norwood, MD[4], Julian Howell, MB BS[5] and Elizabeth Waldie, BSc (Hons)[5] (Presented By: Adrian Dobs)
[1]Johns Hopkins University; [2]Quality of Life Medical and Research Center, LLC, Tucson, AZ, United States; [3]Panhandle Family Care Associates, Marianna, FL; [4]Valley Endocrine and Valley Research, Fresno, CA; [5]ProStrakan, Galashiels, UK

Introduction: Testosterone (T) replacement therapy is recognized as having an important role in improving the symptoms and physiological wellbeing of men with hypogonadism. Gels are a popular form of T administration due to their non-invasive, once daily application. Both gels currently approved in the US are 1% concentration: this study examined a metered dose 2% gel that requires half the volume to be applied for the equivalent T dose of other gels. The primary objective was to demonstrate that the percentage of patients achieving Cavg in the normal range (= 300 and = 1140 ng/dL) on Day 90 was > 75%.
Methods: This study was a multicenter, open label trial in 149 hypogonadal males, aged 18-75 years, having a single morning T < 250 ng/dL or < 300 ng/dL on two consecutive occasions. Patients who had received prior T replacement therapy completed a washout period. Patients applied Fortigel each morning to the inner thighs at a starting dose of 40 mg. The daily dose of T was adjusted within a range of 10mg to 70mg on the basis of total serum T obtained two hours after application on Days 14, 35, and 60. Serum T was also measured at 2 hours on Day 90. 24 hour pharmacokinetic (PK) T profiles were obtained on Days 35 and 90. Safety (physical examination, labs and ECG) was monitored throughout the study.
Results: The predefined criteria for all PK efficacy endpoints were achieved. The primary efficacy endpoint was achieved, as 76.1% of patients had Cavg T levels within the normal range and the overall mean Cavg T level was 442 ng/dL.
Secondary efficacy objectives were also achieved for Cmax on Day 90:

- = 1500 ng/dL: 91.3% of patients
- = 1800 – = 2500 ng/dL: 4.3% of patients
- 2500 ng/dL: no patients

46.3% (69 patients) had at least one AE however only 3.4% of patients withdrew from the study due to AEs, and only three of these were considered related to study drug. The most common AEs were mild and moderate skin reactions (25 patients, 16.8%) with 8% of patients having visible skin changes confirmed by the investigator. These were transient with median duration of 7-14 days. No patient was removed from the study due to elevated hematocrit.
Conclusion: Fortigel™ 2% Gel was shown to achieve physiological levels of T following titration and was well tolerated by patients. Safety was demonstrated with intermittent skin reactions being the most common AE, although the severity of these was mostly mild.

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LEYDIG CELL SPECIFIC CONDITIONAL DELETION OF ALK3

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University of Massachusetts Medical School

Mullerian inhibiting substance (MIS, also called anti-Mullerian hormone, AMH) not only induces regression of Mullerian ducts during male sexual differentiation, but also modulates the steroidogenic capacity and maturation Leydig cells. The actions of MIS in the testis are mediated through a type II ligand-binding receptor (MISRII) and a shared type I receptor. Activin receptor-like kinase 3 (ALK3) is the type I receptor partner for MIS in the Mullerian duct. In vitro, however, ALK2 and ALK6 (BmpR1b) both also interact with the MISRII, thus, it is unclear whether ALK3 is also the type I receptor for MIS in Leydig cells. To identify the signaling partner for MISRII in Leydig cells, we generated Leydig cell specific ALK3 conditional knock out mice by crossing female ALK3 flox/flox with male ALK3 flox/wt Cyp17 cre+ (17 hydroxylase-cre-IRES-GFP) mice, or male ALK3 flox/flox with female ALK3 flox/wt Cyp17 cre+ mice. We compared the expression of steroidogenic enzymes and steroid concentration in Leydig cells of ALK3 flox/flox Cyp17 cre+ and control mice (ALK3 flox/flox Cyp17 cre- or ALK3 flox/wt Cyp17 cre- littermates). We found differences in mRNA expression of steroidogenic enzymes in the ALK3 flox/flox cre+ mice compared to littermates (see Table). Moreover, the serum androstenedione concentration of ALK3 flox/flox Cyp17 cre+ mice was 1.25 fold higher than that in control littermates.

Leydig cell genes	ALK3 flox/flox Cyp17 cre+	Control	p value
P450scc	2.320±0.398	2.255±0.367	ns
P450c17	3.607±1.135	7.267±1.684	0.028
5á reductase type I	0.020±0.007	0.012±0.004	0.002
3 á hydroxysteroid dehydrogenase (3 á HSD)	0.0204±0.015	0.003±0.001	0.02
17â-hydroxysteroid dehydrogenase III (17â-HSD III)	0.048±0.015	0.121±0.041	0.011
3 â- hydroxysteroid dehydrogenase VI (3 â-HSD VI)	0.038±0.013	0.107±0.046	0.009

Collectively, these results indicate that genes indicative of Leydig cell maturation are expressed at lower levels in the ALK3 conditional knock out model.These findings are similar to that of the MIS type II receptor knock-out mice. These data suggest that loss of either receptor component lead to more immature and less differentiated Leydig cells. This study confirms that ALK3 is the specific type I receptor recruited into the MIS receptor complex in the MIS signal transduction pathway to modulate the development of Leydig cells.

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TESTOSTERONE ADMINISTRATION TO ELDERLY HYPOGONADAL IMPROVES THE METABOLIC SYNDROME, C-REACTIVE PROTEIN AND LIVER STEATOSIS

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[1]Bayer-Schering Pharma; [2]Private Praxis Bremerhaven, Germany

Objectives: The metabolic syndrome is associated with lower-than-normal testosterone levels. Conversely, testosterone deprivation induces features of the metabolic syndrome in men. Hepatic steatosis or non-alcoholic fatty liver disease is an important factor in the pathogenesis of insulin resistance and the metabolic syndrome. Liver fat is highly significantly and linearly correlated with all components of the metabolic syndrome. Peptides and cytokines secreted by adipocytes in the visceral compartment may cause a decrease in peripheral insulin mediated glucose uptake and may increase hepatic fat accumulation. The effects of testosterone administration on variables of the metabolic syndrome and on liver functions (AST / ALT) and C-reactive protein were studied.
Design and methods: A large cohort of 122 middle-aged to elderly hypogonadal men (T levels 5.9 – 12.1 nmol/L, aged 34 – 69 years), were treated for 24-30 months with parenteral testosterone undecanoate (TU) restoring their plasma testosterone to the normal range.
Results: Following testosterone administration values of BMI, waist circumference (WC) declined significantly over the first 21 months and then stabilized. Serum cholesterol (chol), LDL, triglycerides (TG) progressively and significantly declined over the first 12 months and then stabilized while levels of HDL progressively and significantly continued to increase. The decline in WC correlated with the decline in BMI and decline in serum cholesterol and triglycerides but not with serum HDL or LDL. Along with the improvements of features of the metabolic syndrome, there was a decline of CRP, AST and ALT over the first 15 months. There were (low) but significant levels of correlation between the declines of BMI, waist circumference, levels of cholesterol, HDL, LDL and triglycerides on the one hand and the declines of AST, ALT and CRP on the other hand.
Conclusion: Restoring plasma testosterone levels to normal in elderly hypogonadal men leads to significant improvements of features of the metabolic syndrome and of liver steatosis. The improvements are progressive over time.

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CYP2A1 INTERACTS WITH 11Â-HYDROXYSTEROID DEHYDROGENASE 1 IN RAT LEYDIG CELLS

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[1]School of Pharmacy, Wenzhou Medical College; [2]The 2nd Affiliated Hospital, Wenzhou Medical College; [3]Population Council and the Rockefeller University

Objectives: CYP2A1 is a P450 enzyme that catalyzes the metabolism of testosterone. CYP2A1 has been reported to be present in rat testis. However, its developmental changes and function have not been well characterized. The purpose of this study was to measure the abundance of CYP2A1 mRNA (Cyp2a1) in the developing testis and Leydig cells, and to examine the effects of its product, 7á-hydroxytestosterone (7HT), on another important enzyme, type 1 11ï•¢-hydroxysteroid dehydrogenase (11ï•¢-HSD1) that interconverts active corticosterone and inactive 11-dehydrocorticosterone.
Methods: Testes were collected at 0, 4, 7, 14, 21, 28, 56 days after ethane dimethanesulfonate (EDS , a toxin to kill Leydig cells. Cyp2a1 was detected by in situ hybridization and real-time PCR.
11â-HSD1 as detected TLC separation and radioactive scanning.
Results: As detected by real-time PCR and in situ hybridization, Cyp2a1 was found exclusively in Leydig cells. CYP2A1 activity in adult Leydig cells was 5-fold higher than those in progenitor or immature Leydig cells. 7HT competitively suppressed 11â-HSD1 oxidase and reductase activities in rat testis microsome with Ki of 1.2 and 2.9 µM, respectively. However, in intact Leydig cells 7HT did not inhibit 11â-HSD1 reductase activity, but stimulated its reductase activity. Thus at 100 nM and higher, 7HT significantly switched oxidase/reductase towards reductase.
Conclusion: The present data suggests that CYP2A1 functionally interacts with 11â-HSD1.
(Supported in part NIH grant HD050570)

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FGF2 AND LH AFFECT ANDROGEN PRODUCTION IN RAT IMMATURE LEYDIG CELLS VIA DIFFERENT INTRACELLULAR PATHWAY

Yechen Xiao, PhD, Xiaokun Li, MD, Huiyan Wang, MD, Dianne O. Hardy, PhD and Renshan Ge, MD (Presented By: Yechen Xiao)
Population Council

Introduction and Objectives: Basic fibroblast growth factor (FGF2) belongs to a family of heparin-binding growth factors and has multiple functions including the regulation of cell proliferation, migration, survival and differentiation. Both FGF2 and luteinizing hormone (LH) have been reported to regulate androgen production in Leydig cells. However, the interaction between these two hormones in the regulation of androgen is unclear. The objective of the present study is to determine underlying intracellular pathway of the regulation of androgen production in rat immature Leydig cells (ILCs).
Method: ILCs were isolated from 35-day-old rat testes and cultured in DMEM/F12 medium with luteinizing hormone (LH, 1 ng/ml) or FGF2(10 ng/ml) both. ERK inhibitor (PD98059) and PI3K inhibitor (LY294002) were also tested. Total androgen (5á-androstane-3á, 17â-diol and testosterone) was measured by RIA.
Results: LH stimulated androgen production in ILCs (667.3±117.7 ng /106 cell vs. 113.4±11.27 in control). However, FGF2 did not (120.5±10.49 ng/106 cell). PD98059 (20iM) inhibited LH-stimulated androgen production (415.3±100.2 ng /106 cell vs. 667.3±117.7 ng/106 cell), suggesting that ERK pathway may play an important role in steroidogenesis in ILCs. FGF2 (10 nM) inhibited LH-stimulated androgen production. LY294002(20iM) stimulated androgen production, which was abolished by the addition of FGF2 (10nM). These data indicate that FGF2 inhibits LH-stimulated androgen production via inhibiting PI3K pathway. (Supported, in part, by grants from NIH RO1 HD050570 and AG030598).

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THE (+)- AND (-)-GOSSYPOLS POTENTLY INHIBIT HUMAN AND RAT 11Â-HYDROXYSTEROID DEHYDROGENASE TYPE 2

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Objectives: Gossypol has been proven to be a very effective male contraceptive. However, clinical trials showed that the major side effect of gossypol was hypokalemia. Gossypol occurs naturally as enantiomeric mixtures of (+)-gossypol and (-)-gossypol. The (-)-gossypol is found to be the active component of antifertility. 11â-hydroxysteroid dehydrogenase 2 (11âHSD2) has been demonstrated to be a mineralocorticoid receptor (MR) protector by inactivating active glucocorticoids including corticosterone (CORT) in rats, and therefore mutation or suppression of 11âHSD2 causes hypokalemia and hypertension. In the present study, the potency of gossypol enantiomers was tested for the inhibition of 11âHSD1 and 2 in rat and human.
Methods: Human and rat testes were used to prepare microsomes. 11âHSD1 and 11âHSD2 were measured by adding radioactive corticosterone and respective cofactors NADP+ (for 11â-HSD1) or NAD+ (for 11â-HSD2) into microsomes and TLC separation and radioactive scanning.
Results: Both (+) and (-)-gossypols showed a potent inhibition of 11âHSD2 with the half maximal inhibitory concentration (IC50) of 0.61 and 1.33 µM for (+) and (-)-gossypols, respectively in rats and 1.05 and 1.90 µM for (+) and (-)-gossypols, respectively in human. The potency of gossypol to inhibit 11âHSD1 was far less; the IC50 was ? 100 µM for racemic gossypol.
Conclusion: The gossypol-induced hypokalemia is likely associated with its potent inhibition of kidney 11âHSD2.

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THE (+)- AND (-)-GOSSYPOLS POTENTLY INHIBIT BOTH 3Â-HYDROXYSTEROID DEHYDROGENASE AND 17Â-HYDROXYSTEROID DEHYDROGENASE 3 IN HUMAN AND RAT TESTES

Hong-Yu Zhou, Master[1], Guo-Xin Hu, Bachelor[1], Xing-Wang Li, Doctor[1], Bing-Bing Chen, Master[1], Ye-Chen Xiao, Doctor[2], Qing-Quan Lian, Bachelor[1], Guang Liang, Doctor[1], Howard H. Kim, Doctor[3], Xiao-Kun Li, Doctor[1], Dianne O. Hardy, Doctor[4] and Ren-Shan Ge, Master[4] (Presented By: Hong-Yu Zhou)
[1]Wenzhou Medical College; [2]Jilin Agricultural University; [3]Weill Cornell Medical College; [4]The Population Council New York

Objectives: Androgen deprivation is commonly used in the treatment of metastatic prostate cancer. The (-)-gossypol enantiomer has been demonstrated as an effective inhibitor of Bcl-2 in the treatment of prostate cancer. However, the mechanism of gossypol as an inhibitor of androgen biosynthesis is not clear. The present study compared (+)- and (-)-gossypols in the inhibition of 3â-hydroxysteroid dehydrogenase (3â-HSD) and 17â-HSD isoform 3 (17â-HSD3) in human and rat testes.
Methods: Human and rat testis microsomes were prepared. 3â-HSD and 17â-HSD3 were measured by adding respective radioactive pregnenolone or androstenedione and cofactors into microsomes and TLC separation and radio-scanning.
Results: Gossypol enantiomers were more potent inhibitors of rat 3â-HSD with IC50s of ~0.2 µM compared to 3~5 µM in human testes. However, human 17â-HSD3 was more sensitive to the inhibition of gossypol enantiomers, with IC50s of 0.36 ± 0.09 and 1.13 ± 0.12 for (-)- and (+)-gossypols, respectively, compared to 3.43 ± 0.46 and 10.93 ± 2.27 in rat testes. There were species- and enantiomer- specific differences in the sensitivity of the inhibition of 17â-HSD3. Gossypol enantiomers competitively inhibited both 3â-HSD and 17â-HSD3 by competing for the cofactor binding sites of these enzymes.

Conclusions: The potent inhibition of 17â-HSD3 by gossypol may be used to effect androgen deprivation for the treatment of metastatic prostate cancer.

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ANDROGENIC ACTIVITY OF ALLIUM CEPA ON SPERMATOGENESIS IN RAT
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Aim: Allium cepa (onion) has a good effect on diseases treatment worldwide and have been used since ancient times as a medicinal and food origin. Recently several reports have shown that onion has high antioxidant activity. As antioxidants have essential effect on sperm health parameters, we investigated the effect of onion's bulb fresh juice in Spermatogenesis cycle in rats.
Methods: Wistar male rat (n=30) were allocated into three groups, control (n=10) and two test groups (each of ten). Animals in test groups were subdivided into groups of 2 that received onion (0.5and1g/kg/day). Fresh onion juice was administered with gavages for 20 consecutive days. Animals were kept in standard condition. On twentieth day, the testes of rats in the all groups were removed and sperm was collected from epididymis and was prepared for analysis.
Results: Serum total testosterone significantly increased in whole test groups (P<0.05) and level of LH significantly increased only in the group that received the high dose of fresh onion juice, (P<0.05), but the level of FSH did not differ between experimental and control groups. The percentage of sperm viability and motility in both test groups significantly increased (P<0.05), but the sperm concentration significantly increased only in the group that received the high dose of freshly extracted onion juice, (P<0.05) It was evident that there was no difference on sperm morphology and testis weights in test groups comparing to control group. The data were analyzed with SPSS software.
Conclusion: In our study 20 g of freshly prepared onion juice has significantly affected the sperm number and percentage of viability and motility; it seems that using 1mg/kg of freshly prepared onion juice is effective in sperm health parameters.

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SAFETY STUDY O F LONG-ACTING PARENTERAL TESTOSTERONE OVER 24-30 MONTHS
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Objectives: To investigate the safety of the administration of long-acting parenteral testosterone undecanaote (TU) to hypogonadal, mainly elderly men.
Methods: 122 men aged 34 – 69 years (mean ï,±SD = 59.5 ï,± 6.0), with baseline testosterone 5.9 – 12.1 nmol/L (mean ï,±SD = 2.7 ï,± 0.5) were treated with parenteral TU. Of these 122 patients, 48 patients were followed for up to 30 months, 27 for 27 months and 47 for 24 months.
Results: Plasma levels of testosterone rose from 9.3 ± 1,7 nmol/L to 18.7 ± 2.1 nmol/L reaching their maximum at 9 months, never exceeding reference values. International Prostate Symptoms Scores decreased significantly over 24 months (p < 0.001), and then stabilized. There were initial fluctuations in prostate volume and values of PSA over the first 12-15 months treatment, then values stabilized at levels of 5-10% higher than baseline. PSA never exceeded 4 ng/mL. Hemoglobin increased significantly (p < 0.001) never exceeding reference values. The hematocrit increased significantly (p<0.001). Both had reached their maximum values after 12 months. Over the 30 month study period, at any time point, nine patients were had a hematocrit above 52%, the upper limit of normal. No specific measures were taken (dose reduction of testosterone, venipuncture). An elevated hematocrit was never found at two occasions in the same patient.
Conclusions; Over a period of 24-30 months testosterone treatment with TU appeared safe but longer and larger scale studies are needed

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STUDY OF THE CYCLICAL CHANGES OF REPRODUCTIVE HORMONES IN SERUM AFTER EJACULATION IN NORMAL ADULT MEN
Kangshou Yao and Junrong Zhang (Presented By: Kangshou Yao)

Aim: To study the cyclical changes of reproductive hormones in serum after ejaculation in normal adult men. Methods: Continuous 10-day abstinence after ejaculation, the productive hormones in serum including FSH LH TT FT SHBG of the ten regular donors of the human sperm bank were examined before and after ejaculation.
Results: The TT and FT did not chang significantly from the first day to the fifth day and begin gradually increasing from the sixth day and reached a peak in the eighth day, then gradually declined. But FSH LH SHBG were not cyclical changes.
Conclusion: There are some cyclical changes in TT and FT, and these changes may correlate to ejaculation.

SPERMATOGENESIS / STEROIDOGENESIS / TESTIS BIOLOGY

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PROTEOMIC ANALYSIS OF PROTEINS INVOLVING IN SPERMIOGENESIS IN MOUSE
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Background: Spermiogenesis is a unique process in mammalian body covering the process from haploid round spermatids to spermatozoa in the testis. It involves formation of acrosome, condensation of nucleus, generation of sperm tail and removal of most cytoplasm as residual body. And its characterization could provide a future reference for studies involving male infertility such as teratozoaspermia and asthenospermia. Compared with well-studied transcriptional level of spermatogenesis protein-level characterization has been limited. The development of mass spectrometric techniques now allows for the large-scale protein-level characterization of spermiogenesis.
Results: Here we report the high-confidence identification of 2116 proteins involving in spermiogenesis using the advanced hybrid linear ion trap (LTQ)-Orbitrap mass spectrometer, in which 299 were testis-specific and 157 were novel. Chromosome distribution analysis of the identified proteins showed paucity of X-linked proteins and significant enrichment of proteins on Chromosome 11, the latter was due to expansion of testis-expressed protein families. And comparison with transcriptional data implied substantial translational regulation during spermiogenesis. Analysis of proteins identified showed that many proteins possibly functioning in unique processes of spermiogenesis. Of about eighty proteins annotated to be involved in vesicle-related events, VAMP4 was selected to study its function in spermiogenesis using in vivo knockdown experiments by injection of small interference RNA (siRNA) into seminiferous tubules. Injection of siRNA against VAMP4 leaded to decreased VAMP4 protein level in spermatids, and caused large amount of acrosome and head abnormalities in sperm from cauda epididymis. The diffused or fragmented vesicles which failed to fuse to a large acrosome structure were frequently observed using transmission electron microscope in sperm from cauda epididymis, which implied the important role of VAMP4 in membrane fusion events in acrosome formation during spermiogenesis.

Conclusion: The phenomenon of translational regulation necessitates the application of proteomics techniques in the studies of spermiogenesis. This high-confidence characterization of proteins involving in spermiogenesis provide an inventory of proteins useful for understanding the mechanisms of male infertility and provide candidates for drug targets for male contraception and male infertility.

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CHRONIC FETAL / POSTNATAL HYPOTHYROIDISM TRANSIENTLY INHIBITS SERTOLI CELL ANDROGEN RECEPTOR EXPRESSION AND CONSEQUENTLY CAUSES A DELAY IN SPERMATOGENESIS

Katja Teerds, PhD[1], Dirk de Rooij, PhD[2], Hans Swarts[3], Anita van Kesteren-Buiting[3], Anne Klimstra, BSc[3], Jaap Keijer, PhD[3] and Eddy Rijntjes, PhD[4] (Presented By: Katja Teerds)
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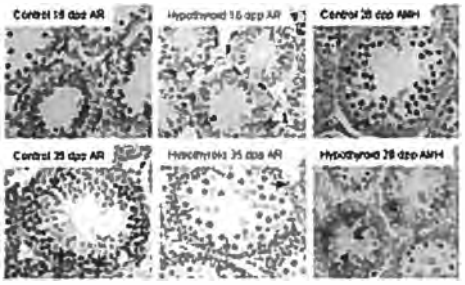
In the present study a relatively mild form of hypothyroidism was induced in rats during fetal development to investigate the effects on testicular development. Dams were fed either an iodide-poor diet to which 0.5% sodium perchlorate was added to deplete endogenous iodide stores, or received a control diet (iodide content according to the AIN-93 guidelines, 0% sodium perchlorate). The hypothyroid diet was continued until the animals were sacrificed between days 16 and 84 postpartum (pp).
The results show that under hypothyroid conditions Sertoli cells continue to proliferate up to at least 28 days pp and tubule lumen formation is first completed by day 42 pp, all suggestive of a delay in Sertoli cell differentiation. At the same time spermatogenesis did not proceed past meiosis, although plasma testosterone levels were 8- to 10-fold higher during this period compared to the age-matched controls. Next, we determined the presence of androgen receptors (AR) in the testis of hypothyroid rats from day 16 to 64 pp by immunohistochemistry. Strikingly we observed that in the 16 day-old hypothyroid testis no AR protein could be detected in the Sertoli cells, while in peritubular/myoid cells the expression seemed unaffected. By day 35 pp some faint immunostaining became visible and by day 42 pp no difference with the controls could detected (Fig. 1). The absence in AR signaling was confirmed by the continued presence of anti-Müllerian hormone (AMH) protein in the cytoplasm of the Sertoli cells up to 35 days pp. Around this age Sertoli cells acquired AR immunoreactivity and meiosis started to progress. By day 42 round spermatids were detected and by day 50 pp elongated spermatids were frequently observed in the hypothyroid testis. By 84 days pp spermatogenesis in the hypothyroid rats had completely normalized, testis weight and tubule diameter were identical with the control animals. Breeding experiments demonstrated that these chronic hypothyroid males were fertile.
The result of this study strongly suggest that chronic hypothyroidism delays the appearance of the AR in Sertoli cells, thus transiently inhibiting spermatogenesis.



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DEVELOPMENTALLY REGULATED ACTIVIN A SIGNAL TRANSDUCTION BY SERTOLI CELLS IS REQUIRED FOR NORMAL MOUSE TESTIS DEVELOPMENT

Catherine Itman, PhD[1], Chris Small, PhD[2], Michael Griswold, PhD[2], Ankur K Nagaraja[3], Martin Matzuk, PhD[3], Chester Brown, PhD[4], Matthias Ernst, PhD[5], David A. Jans, PhD[6] and Kate L. Loveland, PhD[1] (Presented By: Catherine Itman)
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Introduction: Activin A is critical for normal mouse testis development and quantitatively normal sperm production. Activin enhances the proliferation of immature, but not terminally differentiated, Sertoli cells (Boitani 1995). Furthermore, testicular activin production changes during development, being substantially higher in the immature testis relative to the adult (Buzzard 2004, Barakat 2008). In the Inha-/- mouse, chronic excessive activin production causes Sertoli cell-derived tumors (Matzuk 1992) whereas reduced activin bioactivity, in the InhbaBK/BK mouse, delays fertility (Brown 2000). Activin signals are transduced by the transcription factors SMAD2 and SMAD3.
Objectives: To characterize activin signal transduction in Sertoli cells as they develop in an environment of changing activin levels.
Methods: Enriched Sertoli cells prepared from 6 and 15 day old mouse testes were cultured without serum for 24 hours and then treated with 5, 10, 25 or 50 ng/ml activin A. Phosphorylated SMAD2 and SMAD3 in total cell lysates and in nuclear and cytoplasmic fractions were measured by Western blot and SMAD nuclear accumulation was measured by immunofluorescence. Affymetrix microarrays and quantitative PCR were used to identify and measure activin target gene expression in cultured cells and in testes from Inha-/- and InhbaBK/BK mice.
Results: We found that Sertoli cells exhibit developmentally regulated responses to activin A. At lower activin concentrations, immature Sertoli cells exhibit nuclear accumulation of SMAD3, but not SMAD2, although both SMADs are phosphorylated. Higher concentrations of activin induced maximal SMAD3 nuclear accumulation and a small increase in nuclear SMAD2. Terminally differentiated cells exhibited nuclear accumulation of both SMAD2 and SMAD3 with no graded response observed. By microarray and quantitative PCR, we identified novel activin-regulated genes displaying developmental-stage specific expression, coinciding with differential SMAD usage. These include Gja1and Serpina5, required for Sertoli cell development and male fertility (Uhrin 2000, Sridharan 2007). Gja1 and Serpina5 are also modulated by activin in vivo, increased up to 2-fold in Inha-/- testes and decreased by half in InhbaBK/BK testes.
Conclusions: Normal testis development requires carefully regulated activin production and responsiveness with developmentally regulated responses to activin A by Sertoli cells conferred by differential SMAD usage.

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SYNAPTIC DISRUPTIONS, ABERRANT SUMO-1 ASSOCIATION, AND UBIQUITINATION OF ZYGOTENE-PACHYTENE SPERMATOCYTES IN A MAN WITH MEIOTIC MATURATION ARREST: A CASE STUDY

Petrice Brown, MA, PhD[1], Peter Schlegel, MD[2] and Patricia Morris, PhD[3] (Presented By: Petrice Brown)
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Spermatogenesis, the process in which precursor spermatogonia develop into spermatozoa, is the initial step in the process of sperm production. Many components of spermatogenesis are highly susceptible to errors that can lead to failure or reduction in sperm production, resulting in sub-fertility or infertility. Our previous studies show that small ubiquitin-related modifier-1 (SUMO-1) interactions occur during meiosis and synapsis in men. Studies in animals show that numerous gene mutations and aberrant protein interactions often lead to meiotic arrest, particularly in males. We carefully studied an individual who presented with azoospermia associated with biopsy-documented meiotic maturation arrest. Investigation of the testicular tissue obtained show no spermatozoa and most of the spermatocytes arrested at the zygotene-pachytene transition with few observed to be in "true pachytene". Analysis using immunofluorescence microscopy indicated appropriate association of synaptonemal complex protein-3 and kinetochore-associating proteins. When compared with samples obtained from patients with normal spermatogenesis, this patient's spermatocytes displayed prolonged asynaptic regions and possible non-homologous pairing, as indicated by concentrated ã-H2AX staining, typically observed at the sex body of pachytene cells. Aberrant SUMO-1 association was identified in zygotene-like cells localized with synapsed regions of incompletely synapsed chromosomes, in contrast to men with normal spermatogenesis in which SUMO-1 localizes to synaptonemal complex structures only after complete synapses. Spermatocytes from both normal-but-obstructed men and this case individual were further compared using a pan-ubiquitin antibody. No ubiquitin (Ubq) was observed in spermatocytes from control samples at any stage of meiotic prophase. In contrast, essentially all prophase spermatocytes analyzed from the meiotic arrest individual showed significant Ubq nuclear staining throughout the entire nucleus. In summary, this man with spermatogenic arrest at meiosis was shown to have aberrant ã-H2AX, SUMO-1, and Ubq expression in spermatocytes stalled in the zygotene-to-pachytene transition. Taken together, our studies are consistent with an important role(s) for SUMO-1 at meiosis. The altered patterns of sumoylation and ubiquitination suggest a possible pathogenic role(s) of SUMO-1 and Ubq in at least some patients with infertility. Studies supported by NIH R01 HD039024 (PLM).

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DIRECT INTERACTIONS BETWEEN INSULIN-LIKE GROWTH FACTOR BINGDING PROTEIN-3 AND BAX PROMOTES MALE GERM CELL APOPTOSIS IN RAT

Yue Jia, MD, PhD[1], Yanhe Lue[1], Amiya P. Sinha Hikim[1], Laura J. Cobb[2], David Hwang[2], Vince Atienza[1], Pinchas Cohen[2], Ronald S. Swerdloff[1] and Christina Wang[1] (Presented By: Yue Jia)
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Objective: The mitochondria-dependent (intrinsic) pathway, governed by BCL-2 family members, is the key pathway for male germ cell apoptosis across species. Gene microarray analysis showed significant up-regulation of IGFBP-3 after intra-testicular testosterone deprivation in human testes. This study tries to elucidate potential role of IGFBP-3 in the induction of male germ cell apoptosis. Study Design: Groups of 4 adult SD male rats treated with intra-testicular injections with vehicle (Saline) or IGFBP-3 50 ug per testis daily for five days. The effect of IGFBP3 with or without BAX on mitochondrial protein release was measured. ELISA were used to measure IGFBP-3 levels in the lysates. Mitochondrial fractions of testicular lysates were prepared and the interaction between IGFBP-3 and BAX was studied by co-Immunoprecipitation (Co-IP). Dot blotting was used to confirm the IGFBP3-BAX binding in vitro. Results: Germ cell apoptosis, detected by TUNEL assay, increased significantly after IGFBP-3 treatment. Co-IP with BAX antibody, demonstrated binding of IGFBP-3 to BAX in mitochondrial fractions after treatment with IGFBP-3 as compared with controls, accompanied by an increase of germ cell apoptosis. ELISA assays after Co-IP confirmed the increased binding of IGFBP-3 and BAX after IGFBP-3 treatment. Dot blotting studies validated the binding of BAX to IGFBP-3 in vitro. IGFBP-3 induced release of Cytochrome C and DIABLO from isolated mitochondria in vitro. IGFBP-3, at a 4-fold lower dose, when combined with BAX, triggered release of these proteins from mitochondria in vitro. Conclusion: IGFBP-3, via binding to BAX, activates the mitochondria-dependent pathway triggering male germ cell apoptosis. This represents a novel pathway that could have significance for male fertility and testicular disease.

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XENOGRAFTING PROVIDES EVIDENCE FOR A ROLE OF PARACRINE FACTORS IN THE PATHOPHYSIOLOGY OF EQUINE IDIOPATHIC TESTICULAR DEGENERATION

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University of PA, Center for Animal Transgenesis and Germ Cell Research

Introduction and Objective: We previously applied xenografting to study testicular function in stallions and showed that spermatogenesis is reconstituted in normal and cryptorchid horse testes xenografted onto mice. The long-term objective of our current work is to apply xenografting to the study of idiopathic testicular degeneration (ITD) in stallions. ITD commonly causes acquired progressive infertility and sterility in stallions. Endocrinologic studies suggest that ITD is caused by a primary defect in the testis. However this remains to be directly tested. We used xenografting to show that grafting of tissue affected with severe ITD onto the permissive environment of the mouse host did not rescue the ITD phenotype. This confirmed that, in severe cases of ITD, the testis itself, and not the extratesticular environment, is primarily diseased. The objective of our current work is to determine if co-grafting of diseased and healthy stallion testicular tissue will rescue the ITD phenotype. The hypothesis is that paracrine effects from healthy tissue may benefit diseased tissue.
Methods: Fragments of equine testicular tissue were grafted under the back skin of anesthetized castrated male scid mice as described. For each donor, 4 mice received 4 grafts of ITD tissue and 4 grafts of normal, prepubertal testicular tissue with all tissue physically separate. An additional 4 mice received 8 paired grafts in which ITD tissue was placed in physical contact with normal prepubertal tissue. Comparable tissue pieces were fixed at the time of grafting as reference points. Grafts were analyzed at bimonthly intervals for overall condition (number of seminiferous tubules), status of maturation (seminiferous tubule diameter), and spermatogenesis (most advanced germ cell type).
Results: Tissue from ITD donors degenerated to some degree following grafting. But the progression of degeneration in ITD tissue grafted in contact with healthy tissue was delayed compared to that in tissue grafted separately. ITD tissue in physical contact with healthy tissue contained more seminiferous tubules per unit area and more mature seminiferous tubules compared to ITD tissue grafted separately.

Conclusions: These data provide evidence for a beneficial paracrine effect of healthy tissue on ITD tissue and so suggest a role for paracrine factors in the pathogenesis of ITD in stallions.
Supported by a grant from the Grayson Jockey Club Research Foundation and a Firestone Research Award.

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ACTIVIN SIGNALLING MODULATORS IN NORMAL, GONADOTROPIN-DEPRIVED, AND NEOPLASTIC HUMAN TESTIS

Vinali Dias[1], Ewa Rajpert-De Meyts[2], Mai A. Sarraj[3], Rob McLachlan[3] and Kate L. Loveland[1] (Presented By: Vinali Dias)
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Introduction and Objectives: Activin is a pleiotropic growth factor that is a member of the Transforming Growth Factor â (TGFâ) superfamily of signaling molecules. Regulated activin signaling is known to influence several steps in male gamete differentiation. We recently identified Sertoli cells, spermatogonia and spermatocytes as activin signaling targets in human testis, based on their selective production of three activin receptor subunits, ActRIIB, ALK2 and ALK4. We also observed upregulation of the fourth activin receptor subunit, ActRIIA, as pre-malignant carcinoma in situ (CIS) cells progress to form malignant germ cell seminomas. Given the importance of regulated activin signaling in testis development and function, we sought to investigate the cellular sites of production of activin signaling modulators in normal and dysfunctional adult human testes samples.
Methods: Activin signaling transducers (phosphorylated Smad2/3) and antagonists (inhibin á, betaglycan, Smad6, MAN-1) were detected in Bouins fixed, paraffin embedded adult human testis sections using immunohistochemistry. Additional samples examined were from testicular carcinoma patients and from normal men subjected to hormonal suppression with androgen-based contraceptives.
Results: In normal and gonadotropin-deprived testes, all antigens were readily detected in Sertoli cells. In addition, Smad6 and phosphorylated Smad2/3 were observed within spermatogonia and spermatocytes, MAN-1 within spermatogonia, and betaglycan in round spermatids. A strong Smad6 signal was evident in all CIS and seminoma cell nuclei, which also exhibited phosphorylated Smad2/3. Betaglycan and inhibin á were not detected in CIS cells, while MAN-1 exhibited a weak signal. A few seminomas exhibited comparatively strong betaglycan, inhibin á and MAN-1 staining (4 out of 28, 6/28 and 6/28, respectively), although most samples showed low to undetectable signals. The differential expression of betaglycan in testicular carcinomas was verified by in situ hybridization, with betaglycan transcripts detected in all seminoma cells.
Conclusions: Detection of phosphorylated Smad2/3 in all analyzed seminoma samples indicates activin/ TGFâ signalling occurs in tumor cells. The presence of betaglycan, inhibin á and MAN-1 in some tumors indicates these factors may alter activin bioavailability in a subset of cancers.

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PROFILING MICRORNAS IN INFERTILE MEN WITH SERTOLI CELL-ONLY SYNDROME

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MicroRNA (miRNA) is a new class of small, noncoding RNA which can regulate mRNA degradation and translational repression. This study was conducted to identify the profiling of miRNAs and their specific targets differentially expressed in the testes of normal spermatogenesis and Sertoli cell-only syndrome (SCO). Three testicular samples with normal spermatogenesis and 6 samples with SCO were used for study. Total RNA was isolated from the tissues for miRNA array (Agilent microRNA Human v2.). miRNAs with significant differential expressions were identified by the t-test analysis and more than 2-fold changes. A total of four miRNAs were found to be up-expressed in SCO specimen including hcmv-miR-UL70-3p, hsa-miR-136, hsa-miR-630 and hsa-miR-663. Conversely, 12 miRNAs were found to be down-regulated in SCO specimen. Two up-expressed miRNAs, miR-136 and miR-630 and two down-expressed miRNAs, miR-25 and miR-126 were verified by TaqMan quantitative real time PCR. Of the four miRNAs which are up-expressed in SCO specimen, three have been shown to have putative target genes in human. The decreased expressions of these targets in SCO specimen were confirmed by quantitative real time PCR. In conclusion, we have identified four up-regulated miRNAs which are associated with SCO, and the expressions of their target RNAs were significantly decreased. Our results provide the information regarding the roles of miRNAs in regulating gene expression that may ultimately lead to spermatogenic failure.

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LOCALIZATION OF TOPOISOMERASE IIÂ IN RAT GERM CELLS DURING SPERMIOGENESIS

Lihua Liu, MD[1], Benjamin R. Emery, MPhil[2] and Douglas T. Carrell, PhD[2] (Presented By: Lihua Liu)
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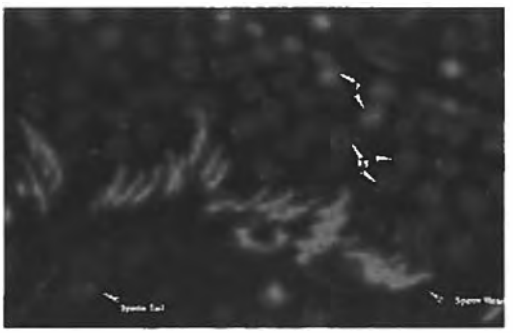
Introduction: Topoisomerase IIâ (TopoIIâ) has been reported to be present in mouse germ cells in a step-specific manner throughout spermiogenesis. It was present in the nucleus of steps 9-13 elongating spermatids (ES). It can also be observed in the cytoplasm of steps 13-15 ES and in the flagella of steps 10-14 ES, but it is absent in round spermatids (RS). It has been proposed to play an important part in chromatin remodeling in elongating spermatids during spermiogenesis. Another study revealed TopoIIâ existed in RS by using Western blot, but they did not show the actual localization inside the RS. Objective: This study is to characterize the localization of TopoIIâ in the rat germ cells during spermiogenesis.
Method: Sprague-Dawley rat testes were sectioned. Immunohistofluoresce was used to stain TopoIIâ, chromatoid body, and Golgi apparatus. Results: In steps 2-8 round spermatids, topoIIâ was observed as a single dot or rod in cytoplasm near nuclear membrane. The topoIIâ stain, which was separate from chromatid body, was proximal to the Golgi apparatus until step 7, which is known to be adjacent to the acrosome. In elongating spermatids, it was present in the nucleus of steps the 9-13 ES. It was also observed in the residual body of step 8 spermatids and in the midpiece of steps 18-19 ES and mature sperm.
Conclusion: The TopoIIâ was present in a step-specific manner throughout spermiogenesis. It is present in the nucleus of steps 9-13 ES like that reported in mouse. Unlike in mouse, it is also present inside cytoplasm of steps 2-8 RS as a single dot or rod and is observed in the residual body of step 19 ES and the midpiece of the mature sperm tail. Further studies are needed in the rat and mouse to identify the localization in detail and to elucidate its function.
Fig. 1 The picture is part of the stage VII seminiferous tubule. The TopoIIâ was stained in red and the nucleus was stained in blue. P: pachytene spermatocyte. RS: round spermatid.



ENVIRONMENT / TOXICOLOGY

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EFFECTS OF PBDES ON ANDROGEN PRODUCTION OF ADULT LEYDIG CELLS

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Population Council

Objective: Polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental contaminants due to their long half-life and widespread use as flame retardants in several consumer products, including plastics. In addition to disrupting thyroid hormone homeostasis, PBDEs may act as developmental neurotoxicants and reproductive toxicants as well. BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) is one of the most abundant congeners found in human breast adipose tissue and maternal milk samples. BDE-47 affected steroidogenesis in vitro, significantly decreasing aromatase activity and estradiol (E2) production at a concentration of 10 μM in H295R human adrenocortical carcinoma cell line, and increasing testosterone (T) secretion in porcine ovarian follicular cells. Our goal in the present study was to evaluate whether low-dose BDE-47 affects the androgen production of Leydig cells and its potential mechanism.
Methods: Adult Leydig cells (ALCs) were purified from the testes of 49-day-old Long-Evans rats, and identified by enzyme histochemical staining for 3â-hydroxysteroid dehydrogenase activity. ALCs were incubated at 34oC in a humidified atmosphere of 5% air: 5%CO2 and treated by different concentrations of BDE-47 (0.01, 0.1, 1, 10 and 100iM) for 24hr, with or without luteinizing hormone (LH, 0.1ng/mL). Phenol red-free DMEM:F12 medium served as the control. In order to explore the potential effect of BDE-47 on steroidogenesis, 0.2 mM 8-Br-cAMP and/or 5 iM 22(R)-hydroxycholesterol were added to the medium and treated ALCs for 1hr. Medium T levels were measured by radioimmunoassay (RIA). All experiments were conducted in duplicate and repeated at least twice.
Results: BDE-47 alone increased T production of ALCs at a dose-dependent manner at concentrations of 10 iM and higher. LH at concentration of 0.1ng/ml significantly stimulated T production. However, BDE-47 did not further increase LH-stimulated T production. 8-Br-cAMP and/or 22(R)-hydroxycholesterol significantly increase T production in ALCs, and BDE-47 showed synergetic increases of T production at concentrations from 10 nM to 10 iM.
Conclusion: Results indicate that BDE-47 increase Leydig cell steroidogenesis. The synergetic effect between BDE-47 and cAMP/22(R)-hydroxycholesterol suggests that BDE-47 affects T production at the level of post-cAMP generation and/or cholesterol side chain cleavage enzyme.
Supported in part by a Bixby Foundation (Y.H.Z.) and NIEHS grant R01 ES10233 (M.P.H./ R.S.G)

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EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE ON RAT LEYDIG CELL REGENERATION

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Population Council and Rockefeller University

Background and Objectives: Di-(2-ethylhexyl) phthalate (DEHP), the most widely used plasticizer, has developmental and reproductive toxicity. It has been demonstrated that pubertal DEHP exposures increased Leydig cell number but decreased steroidogenesis. However, whether the increased number is caused by the increases of proliferation of stem Leydig cell (SLC) and its commitment into progenitor Leydig cell (PLC) is unclear. The goal of the present study is to determine whether there is increase of proliferation of SLC and its commitment into PLC and thereafter long-term effects on the Leydig cell regeneration.
Methods: 90-day-old Long-Evans rats were randomly divided into 3 groups, and were gavaged with the com oil vehicle or DEHP 10 or 750 mg/kg daily for 42 days. All rats received intraperitoneal (i.p.) injection of 75 mg/kg body weight (BW) ethane dimethanesulfonate (EDS) to kill adult Leydig cells (ALCs) 7 days after DEHP treatment. The rats were then killed 11, 21, 28 and 42 days after DEHP treatment. One hour before being killed, the rats were received i.p. injection of 5-bromo-29-deoxyuridine (BrdU) at 100 mg/kg BW. Serum testosterone (T) levels were assessed by RIA. The mRNA expression levels of Leydig cell genes were measured by real-time PCR.
Results: EDS killed ALCs 4 days after EDS treatment as judged by undetectable serum T and undetectable 3â-hydroxysteroid dehydrogenase (3â-HSD) positive cells in the interstitium in control testis. However, in both DEHP treatment groups, there were detectable serum T and some spindle-shaped 3â-HSD positive cells in the interstitium. These 3â-HSD positive cells were not stained by the antibody against 11â-hydroxysteroid dehydrogenase 1 (11â-HSD1), a marker for immature and adult Leydig cells. Real-time PCR detected the disappearance of Leydig cell marker mRNA levels including Lhcgr, Cyp11a1, Cyp17a, Insl3 (ALC marker) and Hsd11b1 in control testes. However, there were detectable levels of Lhcgr, Cyp11a1 and Cyp17a mRNA and undetectable levels of Insl3 and Hsd11b1 in DEHP groups, indicating that these 3â-HSD positive cells were PLCs. The mRNA levels for Nes (marker for SLC) significantly increased in control testes, but not in DEHP treated testes, suggesting that few SLCs were present in DEHP treated testes.
Conclusion: The present study suggests that DEHP stimulates the proliferation of SLC and their commitment to PLCs.

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COMPOUNDING EFFECTS OF LEAD and NICOTINE ON RAT SPERM DNA DAMAGE IN A SURGICALLY INDUCED VARICOCELE MODEL

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Men with varicocele have significantly higher levels of sperm DNA damage than men without varicocele. It has also been shown that lead and nicotine exposure both contribute to sperm DNA damage. However, the contribution of a toxic exposure with varicocele-associated DNA damage has not been determined. We investigated the effects of a surgically induced varicocele with or without lead or nicotine exposure on sperm DNA damage in Sprague-Dawley rats.
A varicocele was surgically induced by partially occluding the left renal vein. Experimental rats were exposed to either 6% lead acetate in water ad libitum, or 0.25mg/100g QOD nicotine by intraperitoneal injection. Controls had no treatment, or were exposed to either toxicant without the varicocele treatment. Sham operations were used as a surgical control. The terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay was performed on epididymal sperm to determine sperm DNA damage. The Student's T-test was used to determine statistical significance.
The results of the TUNEL assay are described in table format below:
Percent DNA Damage in sperm from Contralateral and Ipsilateral Testes Between Treatment Groups

Treatment	Contralateral	Ipsilateral	p value
C	0.2 ± 0.12	0.48 ± 0.30	0.38
V	1.47 ± 0.55	14.87 ± 6.43	0.06
L	0.55 ± 0.17	4.66 ± 1.98	0.04*
LV	1.32 ± 0.44	14.06 ± 3.79	0.002*
N	0.16 ± .011	0.05 ± 0.05	0.43
NV	3.25 ± 1.49	32.04 ± 6.72	0.0005*

Between Groups		
C vs V	0.48 ± 0.30	14.87 ± 6.43
0.02*		
C vs L	0.48 ± 0.30	4.66 ± 1.98
0.12		
C vs LV	0.48 ± 0.30	14.06 ± 3.79
0.008*		
L vs LV	4.66 ± 1.98	14.06 ± 3.79
0.03*		
C vs N	0.48 ± 0.30	0.05 ± 0.05
0.19		
C vs NV	0.48 ± 0.30	32.04 ± 6.72
0.0002*		
N vs NV	0.05 ± 0.05	32.04 ± 6.72
0.0003*		

C = control; L = lead; LV = lead + varicocele; N = nicotine; NV = nicotine + varicocele; * = statistical significance

These data show that the effects of varicocele on sperm DNA damage are compounded by exposure to either lead or nicotine. Interestingly, lead exposure alone appears to affect sperm in the ipsilateral testes more than the contralateral testis, although exposure combined with a varicocele compounds its affects. Nicotine exposure in combination with varicocele did induce a highly significant increase in DNA damage.

PROSTATE / TESTIS CANCER / CLINICAL UROLOGY

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REAL-TIME PERIPROSTATIC TISSUE IMAGING WITH MULTIPHOTON MICROSCOPY FOR IMPROVING POTENCY OUTCOMES DURING NERVE-SPARING RADICAL PROSTATECTOMY: INITIAL RESULTS FROM FRESH HUMAN PROSTATECTOMY SPECIMENS

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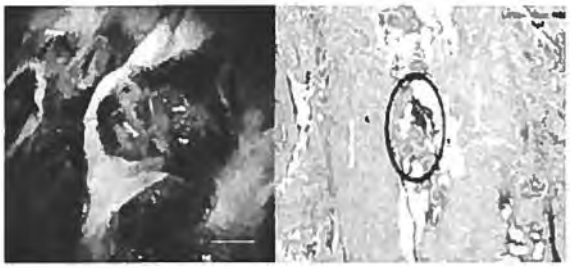
Aim: Multiphoton microscopy (MPM), combined with second harmonic generation (SHG), is a novel technology that permits bioimage acquisition using several low-energy photons to induce autofluorescence of cellular components without use of exogenous stains nor damage to live tissue. With promising results established in our adult male Sprague-Dawley rat model, we now report our initial experience with fresh human prostatectomy specimens.

Patients and Methods: 16 fresh ex vivo human prostatectomy specimens were imaged under an Olympus X61WI upright fluorescence microscope. A femtosecond pulsed Titanium/sapphire laser at 780nm wavelength was used to excite the periprostatic cellular tissue. SHG signals were collected at 390 (+/-35) nm and autofluorescence registered at 380-530nm. Tissues were labeled and correlated with final images obtained at HandE histopathologic confirmation. Institutional review board approval was obtained prior to study commencement.

Results: High-resolution images of the prostatic capsule, periprostatic vessels, smooth muscle cells, and periprostatic inflammation were documented. Histopathologic confirmation of these structures with HandE was closely congruent with images obtained at MPM.

Conclusions: Multiphoton microscopy with SHG delivers superior real-time high-resolution cellular bioimages. Our pilot feasibility study demonstrates the potential for improving potency and cancer clearance outcomes during radical prostatectomy through augmented real-time visualization / preservation of the periprostatic structures with eventual integration of the technology into laparoscopic and robotic platforms.



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THE NATURAL HISTORY OF SMALL INCIDENTAL HYPOECHOIC TESTICULAR MASSES IN INFERTILE MEN: IS SURVEILLANCE THE NEW STANDARD OF CARE?

Matthew Roberts, MD, Paul Toren, MD, Irene Lecker, Ethan Grober, MEd, MD, Keith Jarvi, MD and Kirk Lo, MD (Presented By: Matthew Roberts)
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Introduction and Objectives: With the widespread use of scrotal ultrasound in the evaluation of male infertility, the incidence of small, incidentally detected testicular masses is increasing. Traditionally, treatment has been surgical removal due to concerns about malignancy. Ultrasound follow-up has been proposed as an alternative to surgery. We present the largest series to date of small, non-palpable, incidentally detected testes masses, and document the natural history of these lesions.

Methods: We reviewed the records of patients seen at the Mount Sinai Hospital Fertility Clinic from 2003-2008. Patients with one or more testicular lesions fitting all of the following criteria were included in the study: intratesticular, hypoechoic, diameter < 1cm, non-palpable. We assessed patient age and semen parameters, the size and growth of the lesion(s) on serial ultrasounds, need for surgery, and pathologic diagnosis.

Results: Of 4418 patients evaluated, 46 (1% of total) met the inclusion criteria. Mean age was 35. Semen analysis showed azoospermia, oligospermia, and normospermia in 15, 18, and 7 patients respectively, and was unavailable in 6 patients. Mean follow-up interval was 253 days, and mean number of ultrasounds was 2.8 (range 1-7). Mean lesion diameter was 4.4 mm (range 1-10mm) and there was no significant change in mean diameter over time. Of the 46 patients, 38 had serial ultrasound follow-up only, 3 had immediate surgery, and 5 had surgery following a period of ultrasound follow-up. Indications for surgery were interval growth in 2 (1 partial, 1 radical orchiectomy) and patient choice in 6 cases (partial orchiectomy). One patient had radical orchiectomy for pure seminoma identified due to interval growth from 3 mm to 6 mm at the 3 month ultrasound. The other 7 lesions excised by partial orchiectomy were benign (Leydig cell tumor in 5, unspecified in 2).

Conclusions: In this large series of infertile men, the vast majority of small, non-palpable testicular masses were safely followed with serial ultrasound and did not show significant growth or require surgical removal. 1 patient underwent radical orchiectomy for pure seminoma and remains recurrence-free. All other patients undergoing surgery had benign lesions. Serial ultrasound follow-up of small, non-palpable, hypoechoic testicular masses detected incidentally during work-up for infertility appears to be a safe alternative to immediate surgical removal.

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MOLECULAR INTERACTIONS BETWEEN GALECTIN-3 AND PROSTATE SPECIFIC ANTIGEN (PSA) SECRETED BY PROSTATE CANCER CELL LINES

Rebecca Gilbride, BS, Ashley Block, BS, Sarika Saraswati, BS, MS, PhD and Alan Diekman, BS, PhD (Presented By: Rebecca Gilbride)

Prostate specific antigen (PSA) is a serine protease that is secreted by prostatic epithelial cells, is used as a biomarker for prostate cancer, and is implicated in the promotion of localized prostate tumors and bone metastases. Galectin-3 is a multivalent, carbohydrate-binding protein involved in immunomodulation, apoptotic regulation, angiogenesis, and metastatic cell adhesion in multiple cancers, including prostate cancer. The galectin-3 protein is composed of a carbohydrate recognition domain and a non-lectin binding domain separated by a collagen-like linker sequence. Galectin-3 has been shown to regulate protease function via its interaction with carbohydrate moieties on target protease ligands, while galectin-3 function is regulated by proteolytic processing that prevents galectin-3 multivalency. Previous results from our laboratory demonstrated that PSA from human semen proteolytically cleaved galectin-3. In the current study, PSA was enriched from the culture supematants of LNCaP and C4-2 prostate cancer cells by thiophilic-gel chromatography. Column fractions containing PSA were identified by anti-

PSA immunoreactivity, and PSA-containing samples from the LNCaP and C4-2 supernatants proteolytically cleaved galectin-3 in an in vitro cleavage assay. The proteolytic activity was inhibited by the serine protease inhibitor phenylmethylsulphonyl fluoride. These results implicated PSA secreted by prostate cancer cells in the regulation of galectin-3 function by proteolytic cleavage. Based on reported carbohydrate structures, the non-sialylated N-linked glycans on PSA from LNCaP cells are excellent candidates for galectin-3 binding ligands. Furthermore, characterization of PSA secreted by LNCaP and C4-2 cells by two dimensional electrophoresis analysis demonstrated a similar isoelectric point for LNCaP and C4-2 PSA which was higher than that of seminal plasma PSA, suggesting C4-2 PSA may have a non-sialylated, N-linked glycan similar to the LNCaP PSA N-glycan. To simulate the carbohydrate structure of PSA from prostate cancer cells, PSA purified from human seminal plasma was treated with sialidase. Lectin blot analysis demonstrated that galectin-3 can bind to desialylated PSA but not to sialylated PSA, suggesting that galectin-3 may bind to PSA secreted by prostate cancer cells. Further investigation is warranted to test the hypothesis that PSA function in prostate cancer progression is exerted and/or regulated by molecular interactions with galectin-3.

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RELATIONSHIP BETWEEN NON-ENZYMIC ANTIOXIDANT PROFILE AND MEAN PROSTATE SPECIFIC ANTIGEN (MPSA) LEVELS OF KNOWN PROSTATE CANCER PATIENTS

Oluyemi Akinloye, PhD[1], Olatunji I. Kareem, FMLSCN[2] and Oluwatosin A. Adaramoye, PhD[3] (Presented By: Oluyemi Akinloye)
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Oxidative stress has been implicated in the etiology of several pathologies, prostate enlargement inclusive. The present study was designed to relate the non-enzymatic antioxidant levels in the prostate cancer patients with their mean prostate specific antigen (mPSA) values. Consented patients were recruited from the Cancer Screening Unit (CSU), University College Hospital (UCH), Ibadan. A total of 120 prostate cancer patients were assigned into three groups based on their mPSA values; group 1 with mPSA of6.5µ/L, group 2 and 3 with mPSA of 15.9µ/L and 73.8 µ/L, respectively. Patient had no recent hormone therapy and/or radiation therapy. Likewise, 120 aged matched, apparently healthy subjects were recruited as controls. The study received the approved of the Oyo state government ethical committee. Patients with mPSA between 6.5µ/L to 73.8 µ/L had significantly lower serum uric acid and vitamin E level ($p<0.001$) than the controls. Significantly reduction ($p<0.001$) in serum vitamin C levels were also observed in patients with mPSA of 15.9 µ/L to 73.8 µ/L when compared to the control. Precisely, serum vitamin C levels were decreased by 32% and 47% respectively. The extent of lipid peroxidation (LPO) in the sera of patients was estimated by measuring the thiobarbituric acid reactive substances (TBARs) formed. Serum LPO was significantly elevated ($p<0.001$) in patients with mPSA of 6.5 µ/L to 73.8 µ/L compared to the control. Specifically, LPO was elevated by 28%, 35% and 46% in patients with mPSA of 6.5, 15.9 and 73.8 µ/L, respectively. Furthermore, serum selenium levels were decreased by 35%, 34% and 38% in patient with mPSA of 6.5, 15.9 and 73.8 µ/L, respectively. These results indicate an inverse relationship between the non-enzymatic antioxidant profile of the prostate cancer patient and their respective mPSA values. This relationship should not be overlooked and may serve as basis to further understand the biochemical aspect of the disease.

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OLD MARKERS WITH NEW MEANINGS FOR PROSTATE CANCER

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With PSA screen extensively used, detection of prostate cancer becomes easier than other malignant diseases, but only PSA could not perfectly evaluate the risk of prostate cancer. We report a rare case of primary adenocarcinoma of the prostate producing old tumor markers: carcinoembryonic antigen (CEA), human growth hormone (HGH) and carbohydrate antigen 125 (CA125). A 50-year-old man referred to our hospital with dysbasia. His serum PSA level was 82.37ng/ml (normal <4ng/ml), and his serum CEA, CA125 and HGH levels were 73.53 ng/ml (normal <5.00 ng/ml), 214.87ku/L(normal <35.00) and 11.83 ng/ml(normal <7.50) respectively. Multiple lesions were found in ECT bone scan, but digital rectal examination was normal and twelve-core prostate biopsy was negative. Metastasis in bone marrow could be found PSA positive by immunohistochemical staining. This patient was not sensitive to maximum androgen blockade therapy, the disease rapidly progressed and he died eight months later. Serum CEA, HGH and CA125 level probably give more prognostic information for prostate cancer patients.

GENETICS

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A NOVEL MOUSE MODEL OF MALE INFERTILITY – A LINK TO LUNG CANCER

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The establishment of male infertility is a multi-step process and it has been estimated that these processes encompass the coordinated activation of >1,500 testis genes. Theoretically mutations in any one of these genes could lead to infertility. In order to identify genes critical to male fertility, we have screened N-ethyl-nitrosourea (ENU) mutagenised mice for male infertility phenotypes. We have identified a novel model which we have designated "Joey". Affected Joey males are outwardly normal, but are sterile, whereas affected female mice have normal fertility. Spermatogenesis of affected Joey mice appears normal up to the step 8 of haploid germ cells development, after which it ceases and germ cells are lost via sloughing. The timing of this arrest is consonant with the initiation of sperm tail development and the condensation of the haploid nucleus.
The causal mutation resulting in a conserved amino acid substitution in a gene encoded RNA binding protein designed "Joey RNABP" has been recently identified. A large number of RNA binding proteins have been shown to play key roles in RNA in post-transcriptional control which can occur at many different steps in RNA metabolism including splicing, polyadenylation, mRNA stability, mRNA localization and translation. In addition to our data showing a role in male fertility, the human orthologue of the Joey RNABP gene is located within the 370 kb overlapping lung cancer homozygous deletion region at 3p21.3 which is one of the most common and earliest events in lung cancer pathogenesis. In vitro data has suggested that the human orthologue of Joey RNABP is a tumor suppressor gene with a role in the initiation and/or progression of lung cancer. Based on the onset of the Joey phenotype, the high incidence of testis-enriched transcripts of widely expressed genes, and the critical dependence of spermatogenesis on translation delay, we hypothesize that Joey RNABP mutant allele results in an hypomorphic protein and that Joey RNABP has an essential role in RNA splicing or in the timing of protein translation. The role of Joey RNABP in regulating spermatogenesis and lung cancer progression is current being investigated.

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REACTIVE OXYGEN SPECIES AND SPERM MITOCHONDRIAL DNA MUTATIONS IN INFERTILE PATIENTS

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[1]Dept. of Anatomy, AIIMS; [2]Dept. of Urology, AIIMS

Introduction: Excess production of reactive oxygen species (ROS) and low antioxidant defense establishes oxidative stress (OS) in the semen. Mitochondria are suspected to be both source and target of ROS. Mutation in mitochondrial genome can impair motility and function of mature spermatozoa.
Objective: The present study was aimed to correlate the oxidative stress and mtDNA mutations in idiopathic infertile men.
Methods: Study includes 33 idiopathic infertile men and 30 fertile controls attending AIIMS, New Delhi, India. Complete semen analysis was performed according to WHO criteria (1999). ROS in the semen was measured by chemiluminescence assay. Superoxide dismutase (SOD), Catalase, and Glutathione peroxidase in the seminal plasma were estimated by standard protocol methods. Whole sperm mtDNA was sequenced by standard PCR-DNA sequencing method.
Results: Infertile group showed significant difference in the sperm count (68.2±18.9 Vs 12.7±4.2), percent sperm motility (70±20.5 Vs 18±4.5) and percent normal morphology (79.5±15.7 VS 8.5±1.7) compared to control men. Infertile group showed significantly ($p < 0.001$) higher ROS levels (185.90+52.83 cpm) /106 spermatozoa compared to fertile controls (5.89+0.92 cpm) / 106 spermatozoa. Catalase and GPx activity in the seminal plasma of the infertile group were found to be significantly lower compared to the control group, but no significant difference was found in SOD level between the two groups. mtDNA sequencing revealed that 66% of the infertile group harbored one or more nucleotide changes (ATPase 6 (nt 9098), ATPase8 (nt 8394, 8701, 8860, 8879), ND2 (nt 4769, 5400), ND3(10165, 10172, 10207, 10398, ND4 (nt 11719), and ND5(nt 12705, 13707, 13708, 13946) in the mitochondrial genome compared to control men inspite some common nucleotide changes (A750G, A4769G) in both the groups.
Conclusions: Higher ROS and low antioxidant enzyme levels in the semen infertile men compared to the controls may be due to large number of nucleotide changes in the mtDNA. Since imbalanced antioxidant levels due to increased ROS could in turn affect DNA (mt and nuclear) integrity of spermatozoa, screening mtDNA mutations in infertile men with severe oxidative stress may help in the better management of infertile men for treatment/ART

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DYNAMICS OF PRE ZYGOTIC AND POST ZYGOTIC SEX RATIO

Ashutosh Halder, MD, DNB, DM (Presented By: Ashutosh Halder)
Associate Professor

Introduction: Studies all over the world suggest a sex ratio at birth of about 943-952 females per 1000 males in the absence of any recognized pressure. Low sex ratios have been recorded in all continents and almost all countries. It is now a well established law of nature that the males exceed females at the time of birth in almost everywhere in the world and it is reasonable to believe biological reason for excess male at birth. Some explanations could be Y preference at fertilization and/or selective elimination of female pre-embryo/embryo
Objective: To find out natural Y (male) preference at fertilization through XY FISH on ejaculated sperm and early preimplantation embryo
Material and Methods: Sexing of ejaculated sperm (human) and preimplantation embryo (human and mouse) by XY FISH
Results: We found skewed sex ratio (table) in ejaculated human sperms (more X bearing sperm) and in mouse and human preimplantation embryos (more male i.e., XY)
Conclusion: Y bearing sperm has preferential fertilization advantage despite low number and more aneuploidy. More male in normal early pregnancy arises through preferential fertilization by Y bearing sperms
Table showing details of XY FISH on spermatozoa and preimplantation embryos

Parameters	FISH Results	Sex Ratio (M:F)	Remarks
XY Sperm FISH	N=11002	1M:1.09F	Skewed sex ratio towards female
X Bearing Sperms	5744 (52.2%)		More X bearing sperm
Normal	5723 (52%)		4X more aneuploidy with Y bearing sperm
Abnormal	0021 (0.2%)		
Y Bearing Sperms	5258 (47.8%)		
Normal	5191 (47%)		
Abnormal	0067 (0.8%)		
XY1 Sperm FISH	N=4506	1M:1.13F	Skewed sex ratio towards female
X Bearing Sperms	2393 (53%)		More X bearing sperm
Normal	2388 (52.9%)		9X more aneuploidy with Y bearing sperm
Abnormal	0005 (0.1%)		
Y Bearing Sperms	2113 (47%)		
Normal	2073 (46.1%)		
Abnormal	0040 (0.9%)		
XY FISH on Mouse Preimplantation Embryo	N=161	1.27M:1F	Skewed sex ratio towards male
Male (XY) including 1 with XYY	90 (55.9%)		
Female (XX)	71 (44.1%)		
XY FISH on Human Preimplan-tation Embryo	N=14		Skewed sex ratio towards male (sample size is too little to conclude)
Male (XY)	11		
Female (XX)	03		

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MITOCHONDRIAL DNA MUTATION ANALYSIS IN SEMEN AND BLOOD SAMPLES OF INFERTILE OLIGOASTHENOZOOSPERMIC (OA) MEN

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Introduction: Sperm mitochondria play a key role in the production of energy (ATP) for sperm motility. Since arrangement of mitochondria in spermatozoa is a complex and unique process, the susceptibility of mitochondrial DNA (mtDNA) to the toxic environment is more than in somatic cells. Objective: The aim of our study was to know which the better parameter, sperm mtDNA is or somatic mtDNA for screening mutations in OA men since both are from different embryonic origin.
Materials and methods: The study included 50 infertile patients with <20% progressive motile and <20 million sperm/mL in the semen and 50 age and ethnically matched fertile controls (who have initiated a successful pregnancy in the last 12 months). Semen analysis was performed according to WHO (1999) criteria. Both blood and sperm DNA were isolated and sequenced for the mitochondrial genes (ND, Cyt b, CO I, and ATPase) by standard PCR-DNA sequencing protocol.
Results: More number of nucleotide changes was detected in the sperm mtDNA than the blood mtDNA (Table) of the OA patients. An average of 11.1 nucleotide changes were observed in the mtDNA of sperm compared to the mtDNA of the blood (6.6) for 10 nucleotides [7028 (CO I),8279, 8280, 8701, 8860 (ATPase), 12612, 12705 (ND5), 15043, 15226, 15257(Cyt.b)] which showed changes.
Conclusion: Based on our results we conclude that frequency of nucleotide changes are more in the germ cells compared to the somatic cells. This may be due to high suscebility of germ cells towards oxidative stress than somatic cells. Thus molecular screening of germ cell mtDNA is a better diagnostic marker than somatic cells to understand the etiology of oligoasthenozoospermia and counsel these men before they opt for ICSI.
Support: Indian Council of Medical Research and AIIMS, New Delhi.

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ANTIMICROBIAL ACTIVITY OF THE CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PROTEIN

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Cystatin-related epididymal spermatogenic (CRES) protein, a member of the cystatin superfamily of cysteine protease inhibitors, exhibits highly restricted expression in mouse testis and epididymis with an age-dependent pattern, suggesting roles in male reproduction. Previous studies have revealed that other cystatin family members, cystatin 3 and cystatin 11, showed antibacterial activity in vitro. This prompted us to investigate the antimicrobial activity of the CRES protein. Methods: colony forming assay and spectrophotometric method were examined respectively to investigate the effect of recombinant CRES protein on Escherichia coli (E. coli) or Ureaplasma urealyticum (Uu) in vitro. The cysteine residues of CRES protein were mutated to determine whether the antimicrobial activity of CRES is dependent on its disulfide bonds. The active center of CRES was also determined by functional analysis of three overlapping CRES polypeptides started from its N-terminus. Furthermore, the antimicrobial mechanism was also investigated by means of electron microscopy. Results: After incubation of 103 colony forming units (CFU) E. coli with 100ï•-g/L CRES recombinant protein fused with Glutathione-S-transferase (GST) for 4 hours, a substantial loss of CFU was observed, and the effect was dose and time dependent. Furthermore, it took 36 hours for Uu to grow to plateau stage when incubated with GST-CRES recombinant protein, while only 18 hours was needed when incubated with GST. The antibacterial and anti-mycoplasma activity were not impaired when the cysteine residues of CRES protein were mutated, indicating the antimicrobial effect was not dependent on its disulfide bonds. Functional analysis of three overlapping CRES polypeptides started from its N-terminus showed that, the N-terminal 30 residues (NT30) loss the antimicrobial activity completely, while NT60 showed similar activity as full-length CRES protein (NT120). These results indicate the active center of CRES protein resided between the 31th to 60th amino acid residues of its N-terminus. E. coli treated with GST-CRES for 4 hours visualized by scanning electron microscope showed a smooth continuous membrane stricture, suggesting the antibacterial activity of CRES was not accomplished by permeabilization of bacterial membranes. Conclusion: The antimicrobial activity of CRES protein suggests it may be involved in protecting the male reproductive tract against invade pathogens.

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LIPID PEROXIDATION IN TESTIS AND EPIDIDYMIS UNDER INTERMITTENT HYPOBARIC HYPOXIA: PROTECTIVE ROLE OF ASCORBIC ACID

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Hypobaric hypoxia, a predisposing environmental condition at high altitude, encountered by mountaineers and workers, jeopardizes their normal physiological functions. The present study was conducted to evaluate the effects of intermittent hypobaric hypoxia on oxidative stress and the protective role of dietary antioxidant supplementation in testis and epididymis. Ten-week-old male Wistar rats were separated in six groups: 1) Normobaric (Nx); 2) Nx+ physiological solution (Nx+PS); 3) Nx+ ascorbic acid (Nx+AA); 4) IHH; 5) IHH+PS; 6) IHH+AA. Animals subjected to IHH were exposed for 96 h followed by a normobaric conditions for 96 h for a total of 30 days. The controls groups (2 and 5) were injected with doses of PS and treated groups (3 and 6) were injected with doses of AA (10 mg. Kg-1 body weight) at an interval of 96 h. Rats were sacrificed at 30 days. The testis and epididymis were collected to determine lipid peroxide formation, activity and glutathione reductase expression and epididymis sperm count was also performed. The results of this study revealed that intermittent hypobaric hypoxia induced lipid peroxidation, a diminution in glutathione reductase activity in testis and epididymis and a significant decreased in the sperm count. The treatment with ascorbic acid prevented those changes. In conclusion, ascorbic acid is capable of decreasing oxidative stress in testis and epididymis under intermittent hypobaric hypoxia by recovering the glutathione reductase activity.

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A NOVEL AND MICROWOUND SURGICAL PROCEDURE FOR CIRCUMCISION USING THE CHINA SHANG RING

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Background: Circumcision can be recommended for HIV prevention in men. Recently the male circumcision was markedly regarded as important event in China. All kinds of methods for circumcision were performed so many years, but how to complete this procedure as far as short time no cut, no bleeding, no suture, is the tough problem. With the China Shang Ring adventing, the problem could be solved.
Material and Methods: A specially prepared measuring tape is used to measure the circumference of the penis under the level of coronal sulcus at that time the penis is not in erectile / swollen status. After scrubbing with a Betadine solution, the skin is washed off with clear water based antiseptic solution. Pushing the needle along the base of the penis between Colles's fascia and Buck's fascia, the lidocaine were injected while the needle goes ahead and ended at the extended lines of frenulum along the base of the penis. The saturation of lidocaine should be 1.2 ml/cm. At first, the inner ring was putted on the penis. Flip the foreskin over the inner ring and tract downward the foreskin, make coronal sulcus exposed completely. Then fixing the position of inner ring and overturned foreskin, adjusting the length of inside and outside foreskin for symmetry. The outer ring was gently put upon the inner ring, and the first tooth of adjustable buckle was set. Continuing moderately the ring's position was changed, specially the frenulum must be completely protected for allowing the penis in erectile status with tension-free extension, now the second tooth could be fastened tightly. Where after the surplus foreskin around the ring was resected using scissors. Finally the Routine disinfecting and bandaging should be done. When 7-9 days after circumcision operation, the ring should be removed using the sample specially designed ring opener simple method. Follow-up events include pain, edema, wound dehiscence.
Results: The 126 cases were successfully performed Circumcision using the China Shang ring. The rate of pain was 99.2%(125) in 2 hours, 50% (63) after 4 hours, 15.8%(20) after 8 hours, 0.8%(1) after 24 hours. The edema should be disappeared after 2 day completely. The wound dehiscence of 4 cases when whose ring were removed, could be recovered by local timely suture.
Conclusion: The microwound surgical procedure for circumcision using the China Shang ring, was safe and simple to perform, and fit on broad application in large population.

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