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Journal of ANDROLOGY

American Society of Andrology

37th Annual Meeting

April 21–24, 2012
Tucson, Arizona

Program and Abstracts

Periodical Supplement to Journal of Andrology Vol. 33 Issue 2

schedule at a glance

37th ASA Annual Meeting
April 21 – 24, 2012

Andrology Lab Workshop
April 21 – 22, 2012

Basic Science Workshop
April 21, 2012

ASA Special Symposium
April 21, 2012

Epididymis Mini-Workshop
April 24, 2012

ASA 37th Annual Meeting
"Male Reproductive Health: This Generation to the Next"
April 21 – 24, 2012
Hilton Tucson El Conquistador
Tucson, Arizona
Program Chairs: Janice P. Evans, PhD and Ajay K. Nangia, MBBS
Location: Turquoise I, II

FRIDAY, APRIL 20, 2012

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

SATURDAY, APRIL 21, 2012

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

10:00 a.m. – 4:30 p.m. **Exhibit Hall Open**
Location: Turquoise III

7:30 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Turquoise III

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

6:10 p.m. – 6:30 p.m. **Updates from NICHD & NIEHS**
Stuart B. Moss, PhD
NICHD
Jerry J. Heindel, PhD
NIEHS

6:30 p.m. – 6:50 p.m. **Distinguished Andrologist Award**

6:50 p.m. – 7:50 p.m. **EMIL STEINBERGER MEMORIAL LECTURE**
Bedside to Bench: Discovery of Genes that Control Reproduction in Men
William Crowley, MD
Harvard Medical School
(Introduced by: Gail A. Cornwall, PhD)

7:50 p.m. – 9:30 p.m. **Welcome Reception**
Location: Turquoise III

SUNDAY, APRIL 22, 2012

6:30 a.m. – 8:00 a.m. **Past President's Breakfast**
Location: Copper Cactus Room

7:00 a.m. – 4:00 p.m. **Exhibit Hall Open**
Location: Turquoise III

7:00 a.m. – 6:00 p.m. **Registration**
Location: Turquoise Foyer

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
Androgen Receptor Regulation in Prostate Cancer and Other Cells
Donald J. Tindall, PhD
Mayo Clinic
(Introduced by: Paul J. Turek, MD)

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

9:15 a.m. – 10:45 a.m. **SYMPOSIUM I**
Paternal Contributions to Embryo Quality and Reproductive Success
Session Chairs:
Sarah Kimmins, PhD
Michael L. Eisenberg, MD

Father Knows Best: Paternal Impacts of Embryogenesis
Douglas T. Carrell, PhD
University of Utah

Paternal Reprogramming of Metabolic Gene Expression in Offspring
Oliver Rando, MD, PhD
University of Massachusetts

Effects of First- and Second-Hand Tobacco Smoke: How Does It Affect Male Reproductive Function
Francesco Marchetti, PhD
Health Canada, Ottawa

10:45 a.m. – 11:00 a.m. **Quick Break**

11:00 a.m. – 12:30 p.m. **Poster Session I**
Location: Exhibit Hall

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

12:30 p.m. – 2:00 p.m. **MENTORING LUNCHEON SPONSORED BY THE DIVERSITY AND TRAINEE AFFAIRS COMMITTEES**
(not included in registration; tickets required)
Location: Presidio I
"My First 'Real' Job: What is It Like to Be an 'Early Stage' Professional?"
Speakers: Distinguished Panel

schedule at a glance

12:30 p.m. – 2:00 p.m. **Editorial Board Luncheon**
Location: Agave I, II

2:00 p.m. – 3:30 p.m. **Concurrent Oral Sessions**
Oral Session I: New Discoveries on Therapeutic and Diagnostic Tools for Male Reproductive Health
Location: Turquoise I, II
Moderators:
Hari K. Koul, PhD
James F. Smith, MD

Oral Session II: Male Fertility—Spermatogenesis, Tract Biology and Sperm Function
Location: Coronado Ballroom
Moderators:
William Wright, PhD
Thomas Garcia, PhD

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Exhibit Hall

4:00 p.m. – 4:45 p.m. **LECTURE I**
Hormone Replacement Therapy in Men of Reproductive Age
Edward D. Kim, MD
University of Tennessee
(Introduced by: Mark Sigman, MD)

4:45 p.m. – 5:30 p.m. **LECTURE II**
Crosstalk Between Sertoli and Germ Cells – How Does This Lead to Testicular Cell Differentiation?
Leslie L. Heckert, PhD
Kansas University Medical Center
(Introduced by: Kate Loveland, 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: Sunset Point – Outdoor Area
(Rain Location: Presidio I)

MONDAY, APRIL 23, 2012

7:00 a.m. – 3:30 p.m. **Exhibit Hall Open**
Location: Turquoise III

7:00 a.m. – 6:00 p.m. **Registration**
Location: Turquoise Foyer

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Paternal Obesity Affects Offspring Diabetes Risk
Margaret Morris, PhD
University of New South Wales, Australia
(Introduced by: Patricia Martin-DeLeon, PhD)

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

9:15 a.m. – 10:45 a.m. **SYMPOSIUM II**
Future Technologies and Targets for Improving Men's Health
Session Chairs:
Mohit Khera, MD
Polina Lishko, PhD

H2-Gamendazole, A New Non-Hormonal Oral Male Contraceptive: The Road to Clinical Trials
Joseph S. Tash, PhD
Kansas University Medical Center

Gene Therapy, Stem Cells, and Tissue Regeneration for Erectile Dysfunction: Past, Present, and Future
Trinity J. Bivalacqua, MD, PhD
Johns Hopkins University

Cavernous Nerve Regeneration as an ED Therapy
Carol A. Podlasek, PhD
Northwestern University

10:45 a.m. – 11:00 a.m. **Quick Break**

11:00 a.m. – 12:30 p.m. **Poster Session II**
Location: Exhibit Hall

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

12:30 p.m. – 2:00 p.m. **WOMEN IN ANDROLOGY LUNCHEON AND DISCUSSION**
(Not included in registration fee; tickets required)
Location: Agave

2:00 p.m. – 3:30 p.m. **SYMPOSIUM III**
Male Germ Cells – From Their Birth to Their Grave
Session Chairs:
Marie-Claude Hofman, PhD
Andrew Major, BSci

Human Deleted in AZoospermia (DAZ) Gene Family – 600 Million Years in the Making
Eugene Xu, PhD
Nanjing Medical University

The Rules of Transcriptional Regulation of Spermatogenesis: Keep It Simple
Prabhakara Reddi, PhD
University of Virginia

Sertoli Cells: Not Just Nurse Cells But Undertakers Too
Jeffrey J. Lysiak, PhD
University of Virginia

schedule at a glance

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Exhibit Hall

4:00 p.m. – 4:45 p.m. **LECTURE III**
How a Sperm Learns to Be Fertile
Harvey M. Florman, PhD
University of Massachusetts Medical School
(Introduced by: Michael K. Holland, PhD)

4:45 p.m. – 5:30 p.m. **LECTURE IV**
Aging and Benign Prostatic Hyperplasia – What's the Connection?
Jill A. Macoska, PhD
University of Michigan
(Introduced by: Terry R. Brown, PhD)

5:30 p.m. – 6:30 p.m. **ASA Business Meeting Outstanding Trainee Investigator and Trainee Awards**

7:00 p.m. – 11:00 p.m. **Annual Banquet**
(Not included in registration fee; tickets required)
Location: The Last Territory, Hilton Tucson El Conquistador

TUESDAY, APRIL 24, 2012

7:00 a.m. – 8:00 a.m. **2013 Program Committee Meeting**
Location: Boardroom

7:30 a.m. – 12:15 p.m. **Registration**
Location: Turquoise Foyer

8:00 a.m. – 9:30 a.m. **SYMPOSIUM IV**
Androgen Actions and Responsive Tissues
Session Chairs:
Jacques Tremblay, PhD
Johanna Hannan, PhD

Cross Talk of the Androgen Receptor and DNA Damage Pathways: Molecular and Translational Prostate Cancer Relevance
Karen E. Knudsen, PhD
Thomas Jefferson University

Applying "-omics" Technology to Understand Sperm Production
Liza O'Donnell, PhD
Prince Henry's Institute

Dissecting Androgen Action: New Clues from Conditional Knockout Mice
Lee B. Smith, PhD
MRC, Human Reproductive Sciences Unit

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

9:45 a.m. – 10:45 a.m. **INTERNATIONAL LECTURE**
Environmental Perturbations and Vulnerabilities in Male Reproductive Health
Jorma Toppari, MD, PhD
University of Turku, Finland
(Introduced by: Patricia S. Cuasnicu, PhD)

10:45 a.m. – 12:15 p.m. **SYMPOSIUM V**
Genetic Foundations of Male Infertility
Session Chairs:
Kirk C. Lo, MD
Sherwin Zagaroff, MD

Reproductive Fitness of the Human Y Chromosome
Sjoerd Repping, PhD
University of Amsterdam

New Insights in Genetics of Oligozoospermia in Infertile Men
Alexander Yatsenko, MD, PhD
University of Pittsburgh

Mutations in X-Linked Genes as Cause of Infertility in Men
Jeremy Wang, MD, PhD
University of Pennsylvania

MEETING ADJOURNED

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president's welcome

Gail A. Cornwall, PhD

I am pleased to welcome you to the 37th Annual Conference of the American Society of Andrology held at the Hilton Tucson El Conquistador resort from April 21 - 24, 2012. Tucson will be a welcome break for those experiencing cold and gray winter days as April weather is typically sunny and mild with temperatures in the 80s during the day. Derived from the O'odham (Uto-Aztecan language of southern Arizona) name *Cuk Son*, meaning at the base of the black hill, Tucson is in the Sonoran desert surrounded by five ranges of mountains and is the perfect place to enjoy all the raw beauty of the Southwest while attending an intellectually stimulating meeting.

This year's program chairs, Janice Evans and Ajay Nangia, have put together an outstanding program under the theme "Male Reproductive Health: This Generation to the Next." A broad range of topics will be presented including lectures on genes that control reproduction in men, environmental effects on male reproductive health, androgen action, prostate cancer, genetic foundations of male infertility, germ cells and Sertoli cell function, hormone replacement therapy, new therapies for ED, as well as intriguing insights into the father's contributions to reproductive success and how paternal obesity can affect diabetes risk in offspring.

Immediately preceding the ASA meeting are several satellite sessions that all are encouraged to attend. Drs. Allen Seftel and Mohit Khera will again host the highly successful clinical symposium this year entitled "The Science of Couples Sexual Health." The symposium will run the full day on Saturday and will address critical issues including novel and current therapies for testosterone, an update on erectile dysfunction and Peyronie's disease, and a session on female sexual dysfunction. The Andrology Lab Workshop, under the direction of Dean Morbeck, will also be on Saturday and half day Sunday. This hands-on workshop will teach new methods for classifying sperm morphology. The Basic Science Workshop will also be given on Saturday. This workshop themed "Diagnosing Male Reproductive Capacity in the Laboratory" will describe methodologies used to study normal and disrupted spermatogenesis. Consider staying for the Epididymal Workshop which is scheduled for the Tuesday afternoon immediately after the ASA meeting (April 24, 2012). This special meeting will bring together epididymal biologists and scientists from NICHD to discuss the future of epididymal research and to identify critical topics for future funding.

The ASA has a long standing tradition of recognizing its trainees and thus throughout the meeting there are special events designed just for them including a mentoring luncheon, a trainee forum and mixer, and travel and research awards given based on presentations. The ASA meeting is a wonderful opportunity for trainees to meet and discuss their research with established investigators in the field. Finally, fun social events are planned for all attendees including a welcome reception and banquet. Come and enjoy outstanding science in the beautiful southwest.

I look forward to seeing everyone in Tucson!

Gail A. Cornwall, PhD
President, American Society of Andrology

Past Presidents of the American Society of Andrology

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1992 – 1993	Ronald S. Swerdloff
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1994 – 1995	Glenn R. Cunningham
1995 – 1996	Marie-Claire Orgebin-Crist
1996 – 1997	Arnold M. Belker
1997 – 1998	Terry T. Turner
1998 – 1999	Richard V. Clark
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2003 – 2004	Gail S. Prins
2004 – 2005	William J. Bremner
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2006 – 2007	Christina Wang
2007 – 2008	Terry R. Brown
2008 – 2009	Wayne J.G. Hellstrom
2009 – 2010	Dolores J. Lamb, PhD, HCLD
2010 – 2011	Paul J. Turek, MD

**Deceased*

American Society of Andrology

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Allen D. Seftel, MD, FACS; Jacques J. Tremblay, PhD Miles F. Wilkinson, PhD

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	Jay I. Sandlow, MD, Milwaukee, WI (Co-Editor)
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Program Committee	Janice P. Evans, PhD, Baltimore, MD (Co-Chair)
	Ajay K. Nangia, MBBS, Kansas City, KS (Co-Chair)
Public Affairs and Policy Committee	Stephanie T. Page, MD, PhD, Seattle, WA
Publications Committee	Marvin L. Meistrich, PhD, Houston, TX
	Jacques J. Tremblay, PhD, Quebec City, QC, Canada (Co-Chair)
Special Symposium	Allen D. Seftel, MD, FACS, Camden, NJ
	Mohit Khera, MD, Houston, TX (Co-Chair)
Trainee Affairs	George L. Gerton, PhD, Philadelphia, PA
	Peter Liu, MBBS, PhD, Concord, NSW, Australia (Co-Chair)

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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

Tucson boasts the best of both worlds...the progress and innovation of a metropolitan community and the friendly, caring atmosphere of a small town. Tucson's rich cultural heritage centers around a unique blend of Native American, Spanish, Mexican and Anglo-American influences. Blessed with the natural beauty of the Sonoran Desert and endless sunshine, Tucson is the perfect destination for the ASA 37th Annual Conference. Whether you're visiting Tucson for the first time, or you're a local searching for what's new, Tucson has what you're looking for. With incredible attractions for the whole family, plenty of sunshine for outdoor adventures and golf, an extensive arts and culture background, world-class accommodations and spas, and a burgeoning culinary nightlife scene, Tucson will keep you entertained and wanting to come back for more. Come visit Tucson and see for yourself! For detailed visitors information on Tucson, Arizona visit the Metropolitan Tucson Convention & Visitors Bureau at www.visittucson.org.

RECREATION & ACTIVITIES
At the Hilton Tucson El Conquistador Golf & Tennis Resort, you'll find plenty of things to do to keep you occupied. Play golf on their championship golf courses, enjoy a game of tennis, keep fit in the gym, unwind with a specialty spa treatment and more. ASA attendees staying at the Hilton Tucson El Conquistador will receive special rates and discounts on golf and tennis that are included in the complimentary Resort Value Pack. And if you decide to go further afield, Tucson offers a host of attractions and activities right on your doorstep.

SHOPPING & DINING
The Hilton Tucson El Conquistador Golf & Tennis Resort is conveniently located just a short cab ride away from several art galleries, fashion boutiques, and exceptional restaurants.

La Encantada: www.laencantadashoppingcenter.com
Casas Adobes Plaza: www.casasadobesplaza.com
Old Town Artisans: www.oldtownartisans.com
Tubac: azstateparks.com/Parks/TUPR/index.html

WEATHER
Tucson is the sunniest city in the United States! With more than 350 days of sunshine every year, that means there's more time to enjoy all the amazing outdoor activities, events and fun things to do in the Old Pueblo. Tucson in April has an average maximum daytime temperature of 81°F and an average minimum daytime temperature of 50°F. Spring is a pleasant season, with plenty of warm, dry weather.

Registration/Information Desk Hours are as follows:

Friday, April 20, 2012:
2:00 p.m. – 6:00 p.m.

Saturday, April 21, 2012:
7:00 a.m. – 7:00 p.m.

Sunday, April 22, 2012:
7:00 a.m. – 6:00 p.m.

Monday, April 23, 2012:
7:00 a.m. – 6:00 p.m.

Tuesday, April 24, 2012:
7:30 a.m. – 12:15 p.m.

Exhibit Hall Hours are as follows:

Saturday April 21, 2012:
10:00 a.m. – 4:30 p.m.
7:30 p.m. – 9:30 p.m.

Sunday, April 22, 2012:
7:00 a.m. – 4:00 p.m

Monday, April 23, 2012:
7:00 a.m. – 3:30 p.m.

HOTEL INFORMATION
The American Society of Andrology 2012 Annual Conference will be held at the beautiful Hilton Tucson El Conquistador Golf & Tennis Resort in Tucson, Arizona where special room rates have been arranged for meeting attendees.

Hilton Tucson El Conquistador Golf & Tennis Resort
10000 North Oracle Road
Tucson, Arizona, 85704
Phone: (520) 544-5000
Website: http://www.hiltonelconquistador.com/index.cfm

The Hilton El Conquistador Golf and Tennis Resort is located on the northwest side of Tucson, 23 miles (45 minutes) from the Tucson International Airport.

Room Rate: $179.00 plus applicable taxes
Deadline: Wednesday, March 28, 2012
Reservation Line: (800) 325-7832

Room Rate
The negotiated room rate for the 2012 ASA Annual Conference is $179.00 plus any applicable taxes. This rate is for single or double occupancy in a standard room at The Hilton Tucson El Conquistador. The group rate will be available three days prior to, and three days following the official meeting dates based on availability.

Resort Value Pack
ASA Attendees must reserve a room at the Hilton Tucson El Conquistador within the ASA room block to receive the complimentary Resort Value Pack that includes:
- Guest Room High Speed Internet Access
- Fitness Center Admittance
- Country Club Fitness Center Access
- Valet Parking (Gratuity not included)
- Local Phone Calls, up to 59 minutes
- Golf Club rental with purchase of 9 holes of golf at Pusch Ridge
- Purchase 1 hour court time at Tennis Center, get 2nd hour at no charge

Hotel Deadline
The ASA discounted room rate is based on availability and must be booked by **Wednesday, March 28, 2012**. The block may sell out prior to the cut-off date; therefore attendees are strongly encouraged to make reservations early. If the ASA block sells out, or if you make reservations after **March 28, 2012**, you will be charged the hotel's standard rate.

general information

RESERVATIONS
Reservations can be made by calling the hotel reservation department at (800) 325-7832. In order to receive the ASA group rate you must mention the American Society of Andrology 2012 Annual Conference when making your reservation. Reservations can also be made online by visiting the following website: http://www.hilton.com/en/hi/groups/personalized/T/TUSHTHHSOA-20120416/index.jhtml?WT.mc_id=POG. When calling or making your reservation online you must mention the ASA booking code **"SOA"** to receive the group rate. All reservations must be guaranteed with a credit card at the time of booking.

Check-In/Check-Out
Check-in time is 4:00 p.m. and check-out time is 12:00 p.m. If you have early check-in or late check-out requests, you must contact the hotel directly.

Additional Charges
The guest room rate of $179.00 is exclusive of applicable taxes and surcharges, currently at 15.05%. If you have more than two people in your room over the age of 18, there will be an additional charge of $25.00 per person per night. Children under 18 may stay for free in their parent's room using existing bedding. Rollaway beds are available at a charge of $10.00 per night.

Hotel Cancellation Policy
Reservations must be canceled 3 days prior arrival to avoid cancelation penalties. Guests who checkout prior to their reserved checkout date will incur an early checkout fee of $50.00 to their account. Guests wishing to avoid this fee must advise the hotel at or before check-in of any change in planned length of stay.

TRAVEL & TRANSPORTATION

Airport Information
Tucson International Airport (TUS): www.flytucsonairport.com

GROUND TRANSPORTATION

Hotel Shuttle
The Hilton El Conquistador Resort will provide transfer service to/from the airport specifically for the American Society of Andrology 2012 Annual Conference.

Cost for the transfer service is $46.02 ($40.00 + $6.02 tax) per person each way if 2 or less people per vehicle; and $25.31 ($22.00 + $3.31 tax) per person each way if 3 or more people per vehicle. This does not include driver gratuity.

Shared Rides are an option for individuals traveling to and from the meeting alone. The Hilton El Conquistador Resort will be happy to arrange this for those who are interested.

Shared Rides from Tucson International Airport:
The hotel allows for a 15 minute window for guests to share rides from the airport to the hotel.

Shared Rides From The Hilton El Conquistador Resort:
Departures are easy to arrange for shared rides from the hotel to the airport. Departures typically leave the hotel on the hour and the half hour, to allow as many guests as possible to share a ride.

Charges for transportation provided by Hilton will be charged to your guestroom based on the number of people in the vehicle.

If you wish to take advantage of the Hilton transportation option, the **ASA-Transportation Request Form** needs to be completed and emailed to the resort 5 days prior to your arrival. The Request Form is available on the ASA Annual Conference registration website.

Please note: The Hilton El Conquistador Resort transfer service needs to be arranged in advance. Drivers and vehicles are scheduled based on guest arrival/departure information and are not available on-call at the airport.

If you have any questions regarding transportation, please contact Ann Bridges at the ***Hilton El Conquistador Golf & Tennis Resort*** at (520) 544-1210 or via email at ann.bridges@hilton.com.

Taxis
Taxis are located on the commercial roadway on the lower level in front of the terminal. Taxi service from TIA is provided by three companies under contract with Tucson Airport Authority. Rates are $4.50 flag drop, $2.25 each mile, and $22.00 per hour waiting time. There is no extra charge for baggage or more than one person going to the same location. Average one-way taxi fare from the Tucson Airport to the hotel is $65.00 – $70.00.

AAA Airport Cabs LLC: (520) 299-8294
Discount Cabs: (520) 388-9000
Flash Cab: (520) 798-1111
Yellow Cab: (520) 624-6611

Car Rental
Avis Rent-A-Car is the official rental car company for the ASA 2012 Annual Conference. For reservations, please call (800) 331-1600. You must mention the code **J901055** to receive the discounted rates.

Alternate Transportation
Arizona Stagecoach
Website: http://www.azstagecoach.com/
Phone: (520) 889-1000

Parking
Self parking at the Hilton El Conquistador Resort is complimentary; valet parking is $11/day with unlimited in/out privileges.

events & activities

"Imaging of the Infertile Male – A 21st Century Approach and Future Directions"
Date: Saturday, April 21, 2012
Time: 11:45 a.m. – 1:15 p.m.
Location: Agave
Dr. Alukal will review existing modalities for imaging the testis including ultrasound, MRI, and nuclear medicine imaging, discuss the current limitations of these techniques, and present established clinical indications for their utilization. Advances in testis imaging will be outlined, including novel techniques such as MR spectroscopy of the testis and high resolution ultrasound that may prove relevant in the management of male infertility.
Cost: One ticket included in ALW registration; $35.00 for non-ALW registrations. Please sign up for this event on the registration form.

Welcome Reception
Date: Saturday, April 21, 2012
Time: 7:50 p.m. – 9:30 p.m.
Location: Turquoise III
Join us for a welcome reception to connect with friends and colleagues. Admission to the reception is included in your ASA registration fee; however, it is not included if you are only attending the Basic Science Workshop, Special Symposium and/or Andrology Lab Workshop.
Attire: Business casual or casual attire is appropriate
Cost: One ticket included in ASA registration; $25.00 for additional tickets. Please sign up for this event on the registration form.

Mentoring Luncheon Sponsored by the Diversity and Trainee Affairs Committees
"My First 'Real' Job: What is It Like to be an 'Early Stage' Professional?"
Date: Sunday, April 22, 2012
Time: 12:30 p.m. – 2:00 p.m.
Location: Presidio I
Your post-doc is over! Now, you are looking for or have accepted a new, independent position. So many questions!! What is it like to be "on your own"? What new challenges face you? Where do you turn for support and advice? How do you transition from being a mentee to mentor? What are the risks and rewards? A panel of 3–4 early stage professionals from various walks of life (e.g., basic researcher, clinician-investigator, industrial scientist) will discuss these questions and others you may have. Come with your lists of concerns and quiz the panel!
Speaker: Distinguished Panel
Cost: $5.00 for trainees, $10.00 for non-trainees. Please sign up for this event on the registration form.

Trainee Forum and Mixer
Date: Sunday, April 22, 2012
Time: 6:30 p.m. – 8:30 p.m.
Location: Sunset Point – Outdoor Area
Rain Location: *Presidio I*
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.
Cost: Complimentary; all members of the Society are welcome. Please sign up for this event on the registration form.

Women in Andrology Luncheon and Discussion
Date: Monday, April 23, 2012
Time: 12:30 p.m. – 2:00 p.m.
Location: Agave
Cost: $25.00 per person. Please sign up for this event on the registration form.

Annual Banquet
Date: Monday, April 23, 2012
Time: 7:00 p.m. – 11:00 p.m.
Location: The Last Territory, Hilton Tucson El Conquistador
Attire: Casual (Please note: This venue is outdoors and the ground is gravel; comfortable shoes are recommended.)
Grab your boots and saddle up at this year's ASA Annual Banquet! Enter the Last Territory, nestled on the foothills of the breathtaking Santa Catalina Mountains. The Last Territory courtyard is complete with fire pits, picnic tables, a band shell and western décor, while the interior boasts an authentic western bar, patina lanterns and a wood-burning fireplace. Be sure to join us for an evening that includes Western-style grub and a lot of dancing! Everyone is encouraged to wear their cowboy boots and hats for this night out under the Arizona stars!
Cost: $75.00 per person, $35.00 for trainees. Includes dinner and entertainment. Please sign up for this event on the registration form.

message from the program co-chairs

Janice P. Evans, PhD

Ajay K. Nangia, MBBS

We extend to you a warm welcome to beautiful and sunny Tucson for the 37th Annual Meeting of the American Society of Andrology!

The theme of the ASA meeting this year, "Male Reproductive Health: This Generation to the Next," reflects some of the most pressing questions for the present and future of reproductive biology and health in the male. We face a fascinating and changing landscape for male reproductive health issues. With unprecedented changes in the environment in which we live, questions come up about how this environment affects male reproductive health, as well as the offspring that will be produced. With increases in life expectancy, more men are surviving to advanced age and also living longer in these later decades, producing new issues for healthcare related to male reproduction and general health. And finally, we look with a keen eye for how basic science and translational discoveries will make advances in understanding and managing male reproductive health, in the near term and for future generations. The researchers that make up the ASA community are uniquely positioned to tackle these issues. We hope that the meeting activities over the next four days will provide a framework for intellectually stimulating interactions for all andrologists in attendance.

The meeting's presentations reflect the interactive and transdisciplinary nature that is the heart and soul of the society. The Emil Steinberger Memorial Lecture, which kicks off our 2012 meeting, will be delivered by William Crowley, MD, of Harvard Medical School. His talk is titled, "Bedside to Bench: Discovery of Genes that Control Reproduction in Men." Dr. Crowley is renowned for his 25+ years of work in the neuroendocrine and genetic control of GnRH secretion, its impact on gonadotropin secretion, and gonadal physiology and pathophysiology, from studies using animal models, normal and disease models in the human, information from the Human Genome Project and biochemical and cellular approaches. The 2012 AUA Lecturer will be Donald Tindall, PhD, from the Mayo Clinic, who will speak on his work on examining the regulation of androgen receptors in prostate cancer cells and as well as other androgen-regulated cells. Two other lectures will address key health issues in men across their life span: Edward Kim, MD, who will discuss hormone replacement therapy in men of reproductive age, and Jill Macoska, PhD, whose research examines benign prostatic hyperplasia and its association with age. Additionally, one symposium will cover three distinct aspects of the genetic foundations of male infertility, with talks presented by Sjoerd Repping, PhD, Alexander Yatsenko, MD, PhD, and Jeremy Wang, MD, PhD. Another symposium, "Future Technologies for Improving Men's Health," will address advances in male contraception (Joseph Tash, PhD) and cutting-edge treatments for erectile dysfunction (Trinity Bivalacqua, MD, PhD; Carol Podlasek, PhD).

Several presentations highlight the interest in the andrology research community in the science that provides the foundation for long-term advances in reproductive health. The program includes two lectures that will cover new research in sperm and testis biology. Harvey Florman, PhD, will present recent work from his research program on the molecular regulation of sperm function, and Leslie Heckert, PhD, will speak on the development of the testis and how the interactions between somatic and germ cells contribute to testicular cell differentiation. There will be a symposium that highlights the latest work on the progression of male germ cells through their development and to their ultimate demise, with talks by Eugene Xu, PhD, Prabhakara Reddi, PhD, and Jeffrey Lysiak, PhD. The symposium entitled "Cross Talk of the Androgen Receptor and DNA Damage Pathways: Molecular and Translational Prostate Cancer Relevance" will address various actions of androgens throughout the body, with talks by Karen Knudsen, PhD, Liza O'Donnell, PhD, and Lee Smith, PhD.

Finally, with a special nod to the "next generation" part of the 2012 meeting theme, there will be a series of presentations that address recent findings on how a man's lifestyle and environmental exposure can impact his own reproductive fitness and the health of his offspring. We are excited to have Margaret Morris, PhD, of the University of New South Wales, present the Women in Andrology Lecture; her recent work highlights a newly identified risk of the all-too-common problem of obesity, namely that paternal obesity can have effects in his offspring. This will be complemented by a symposium on "The Father's Contributions to Reproductive Success," with talks by Douglas Carrell, PhD, Oliver Rando, MD, PhD, and Francesco Marchetti, PhD. The 2012 International Lecture also fits with this theme; we are very pleased that Jorma Toppari, MD, PhD, of the University of Turku in Finland, is joining us to present his far-reaching work on environmental insults that perturb male reproductive function.

An important feature of the annual meeting is the platform sessions and poster sessions, composed of submitted abstracts. There will be two concurrent sessions of oral presentations selected from abstracts, each with six speakers, one focused on "New Discoveries on Therapeutic and Diagnostic Tools for Male Reproductive Health," and the other on "Male Fertility—Spermatogenesis, Tract Biology, and Sperm Function." Two poster sessions, one on Sunday and one on Monday, will round out the meeting. Both the platform and poster sessions permit delegates to share their most recent research, and are particularly exciting for the trainees. Please be sure to come out and see the work of the up-and-comers in the field of andrology!

The ASA Annual Meeting is significantly enhanced by numerous satellite meetings and supplemental activities. We are extremely grateful to our colleagues who organize these great events! There is something for attendees at all career stages, from trainees and early stage investigators, to established investigators interested in identifying new research directions and collaborations. The Annual Meeting is preceded by three different events: a symposium on "The Science of Couples Sexual Health," organized by Allen D. Seftel, MD, FACS and Mohit Khera, MD; the annual Andrology Lab Workshop, focusing on "Sperm Morphology: Interactive Instruction of a New Rational Classification Method," organized by Dean Morbeck, PhD, HCLD; and a reprise of the very successful Basic Science Workshop, last done in 2010, organized again for 2012 by Kate Loveland, PhD, with the theme, "Diagnosing Male Reproductive Capacity in the Laboratory." The Annual Meeting will be followed by a half-day workshop on "The Epididymis in Infertility and as a Target for Contraceptive Development," on the afternoon of April 24.

Complementing these scientific activities is a selection of events for networking, career development, discussion, and socializing. These include: the Lab Science Forum Luncheon, which will discuss, "Imaging of the Infertile Male—A 21st Century Approach and Future Directions" (this luncheon is also part of the Andrology Lab Workshop); the Mentoring Luncheon (jointly organized by the Trainee Affairs and Diversity Committees), which will have a panel discussion on "My First 'Real' Job: What is It Like to Be an 'Early Stage' Professional?"; the Trainee Forum and Mixer, which includes honoring the winners of the Trainee Travel and International Travel awards; and the Women in Andrology Luncheon, which celebrates the contributions of female investiga-

message from the program co-chairs

tors and clinicians in male reproductive biology and health. We also hope that you'll have some opportunity to enjoy our meeting site, both the resort and the city. The climate and scenery of Tucson should inspire you to enjoy time with your colleagues and friends out and about—on the golf course, on a hike, for a drink by the pool, or dining on an outside patio at the hotel or the nearby retail areas such as La Encantada.

We thank ASA President Gail Cornwall for offering us this special opportunity to chair the organization of the 2012 Annual Meeting; it has been a distinct honor and pleasure to serve the Society in this way. We are grateful for the input and advice from the andrology community as a whole and especially from our Program Committee members, listed below. We offer special thanks to those who were especially helpful with review of abstracts (Janice Bailey, PhD; Bob Brannigan, MD; Terry Brown, PhD; Marie-Claude Hofmann, PhD; Keith Jarvi, MD; Moira O'Bryan, PhD; Darius Paduch, MD, PhD; Jay Sandlow, MD; Jacques Tremblay, PhD; Wei Yan, PhD). Last, but certainly not least, none of this would be have been possible without the support from the ASA Executive Office and WJ Weiser and Associates, especially Debbie Roller and Angela Beltchenko. These two are truly dazzling in their professionalism, work ethic, patience, and sense of humor! We very much appreciate all the help and support that we received in putting this meeting together, and now hope that you all can enjoy the final product here.

Janice Evans, PhD
Ajay Nangia, MBBS

PROGRAM COMMITTEE
Janice P. Evans, PhD; Baltimore, MD (Co-Chair)
Ajay K. Nangia, MBBS; Kansas City, KS (Co-Chair)
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Robert Edward Brannigan, MD; Hinsdale, IL
Terry R. Brown, PhD; Baltimore, MD
Gail A. Cornwall, PhD; Lubbock, TX
Patricia S. Cuasnicu, PhD; Buenos Aires, Argentina
Marie-Claude Hofmann, PhD; Urbana, IL
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Wei Yan, MD, PhD; Reno, NV

emil steinberger memorial lecture award

William F. Crowley, Jr., M.D

William F. Crowley, Jr., M.D., is the Daniel K. Podolsky Professor of Medicine at Harvard Medical School; director of the Harvard Medical School's Reproductive Endocrine Sciences Center, one of NICHD's 12 competitively-funded Centers of Excellence; director of clinical research at Massachusetts General Hospital (MGH); and founder and past chairman of the Clinical Research Forum.

A recipient of an Honors Curriculum BA from Holy Cross College and MD degree from Tufts School of Medicine, Dr. Crowley trained in internal medicine and endocrinology at MGH from 1969 where he has remained on the MGH and Harvard Medical School faculty since.

Dr. Crowley developed a broad-based translational research program that led to the establishment of the Reproductive Endocrine Unit of the MGH Department of Medicine in 1984. His unit pioneered the use of GnRH analogues in humans to treat children with precocious puberty, a therapeutic application that gained FDA approval in 1988. This therapeutic principle of using GnRH agonist-induced pituitary desensitization as a medical treatment is now widely applied to prostate cancer, endometriosis, and uterine fibroids accounting for a >$3.9B annual pharmaceutical market in 2011.

Dr. Crowley also charted the physiology and translated this into the therapeutic application of pulsatile GnRH therapy to induce ovulation in infertile women and complete sexual maturation and fertility in men with isolated GnRH deficiency with absent or delayed puberty. This treatment also led to FDA approval.

Dr. Crowley and his colleagues have now utilized this clinical human disease model of Isolated GnRH Deficiency to discover several novel genes in both the neurodevelopmental pathways of GnRH (like NEFL and Prokineticin 2) and its neuroendocrine signaling pathways (like kisspeptin and Neurokinin B) that control GnRH biology by combining detailed clinical phenotyping with a range of contemporary genetic approaches.

Most recently, they have demonstrated that mutations in these genes not only underlie the rare syndromes of GnRH deficiency but also play a role in more common reproductive disorders such as hypothalamic amenorrhea. This latter *New England Journal* article in 2011 was his 14th original article in that journal.

In addition to these scientific accomplishments, Dr. Crowley and his colleagues have a unique record of training over 75 young physician scientists. Two thirds of these trainees are women; over 80% of these remain in academic medicine funded by peer-reviewed funding on clinical investigators, and half of those more than 10 years out of training are now full professors. Two have been elected as president of the Endocrine Society and four have served on the Endocrine Society Council.

Dr. Crowley received the Fuller Albright Award of the Peripateic Club (1998); the Endocrine Society's Clinical Investigator Award (1998); and NIH's Clinical Research Center Award for Excellence in Clinical Research (2002). In view of his training record, Dr. Crowley was the first male to receive the Mentor of the Year by Women in Endocrinology (2001). He received the Fred Conrad Koch Award (2005), the Endocrine Society's highest scientific honor and the IPSEN Foundation International Prize for Endocrine Research (2007), the highest international juried research award in Endocrinology. Dr. Crowley was named an Honorary Fellow in the Royal Society of Medicine (Ire) (1999) and served as president of the Endocrine Society (2001).

In 1996, Dr. Samuel Their, then president of the MGH, appointed Dr. Crowley as director of clinical research at MGH. In that role, he has built one of the broadest and most robust institutional infrastructure to support clinical and translational research in the country.

Dr. Crowley is also the founder and chairman of the Clinical Research Forum for the first 15 years of its existence. This group of 60 leading U.S. academic health centers account for over 75% of the NIH's extramural research budget. Under Dr. Crowley's leadership, they are collectively focusing on building a national clinical research enterprise by translating basic research advances into more effective and safer therapies for human illness. When Dr. Crowley passed the chair to Dr. Robert Califf of Duke in 2009, the Forum established the William F. Crowley Leadership Award for Academic Medical Centers as an annual award in his honor.

Serono Lectureship Recipients

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

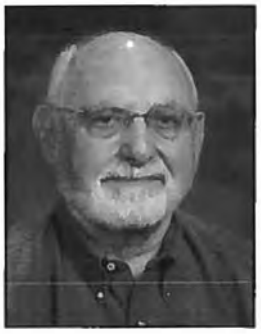
ASA Lectureship Recipients

2004	Judith Kimble
2005	David Page
2006	John R. Aitken
2007	Rudolf Jaenisch
2008	Haifan Lin
2009	Blanche Capel

Emil Steinberger Memorial Lecture Recipients

2010	Andrew Sinclair
2011	Leendert Looijenga, PhD

distinguished andrologist award


Erwin Goldberg, PhD

Erwin Goldberg, PhD, is this year's recipient of the American Society of Andrology Distinguished Andrologist Award. He received his PhD in 1956 from the State University of Iowa with a major in zoology and a minor in biochemistry. After short stays at West Virginia and North Dakota State Universities, Dr. Goldberg joined Northwestern University as an assistant professor in 1963 and has risen through the ranks to his present position of professor of biochemistry, molecular biology, and cell biology. In 2005, Dr. Goldberg received an Honorary Doctor of Science Degree from the State University of New York at Binghamton.

Dr. Goldberg has been an active member of the ASA since 1976 serving on the Future Meetings Committee (1978–1981), Awards Committee (1991–1994), Program Committee (1994-1996), Program Committee for the Post-Graduate Course (1999), and as program chair for ASA Meeting (2002), chair of the Local Arrangements Committee (2003) and chair of the Future Meetings Committee (2003–present). He has served on the editorial boards of the *Journal of Andrology* (1989–1992) and was a candidate for vice president of ASA in 2003. He presently serves on the Board of Reviewing Editors for *Biology of Reproduction*, has been a consultant and reviewer on NIH Study Sections and Special Emphasis Panels since 1982, and a member of Review Boards for the Alzheimer's Association; the Lalor Foundation; CONRAD; and the March of Dimes Birth Defects Foundation.

Dr. Goldberg has had a highly productive career of over 48 years, studying enzymes and proteins unique to mammalian spermatogenesis. Much of his early research has focused on the biochemistry of the germ cell specific isozyme, lactate dehydrogenase (LDH). His original report about LDH in 1963 was a single author *Science* paper and was the first demonstration that there were testis-specific isozymes. This formed the foundation for many later studies that have spanned classical enzyme kinetics, structural analysis of the crystallized protein, and the regulation of the gene. Dr. Goldberg's studies of this gene have been a model for studying transcriptional regulation in the germinal epithelium, and indeed testis-specific transcription in general. Additionally, his laboratory developed and studied mice with null mutations for this isozyme, determining that they are infertile, and he showed that animals could be rescued with the human form of the isozyme. In addition to his basic science studies, he has also been credited for numerous pioneering studies in the field of immunocontraception. In particular, Dr. Goldberg used LDH-C as an antigen in the development of an immunocontraceptive technology for females. Thus, well before "translational research" became fashionable, Erwin was conducting translational research.

Dr. Goldberg has more than 180 scientific publications and has had over 35 consecutive years of NIH funding. Along the way, he has trained 20 PhD students, 10 research Fellows and dozens of undergraduates. Dr. Goldberg also has collaborated extensively with scientists throughout the world. He has even been described as the "Godfather of male germ cell biology."

In summary, Dr. Goldberg has been a pioneer in male germ cell biology and immunocontraceptive. His numerous contributions to reproductive biology over many years make him an outstanding recipient for the ASA's Distinguished Andrologist Award.

Distinguished Andrologists

1976	Roy O. Greep & M.C. Chang
1977	Robert E. Mancini
1978	Robert S. Hotchkiss
1979	Thaddeus Mann
1980	John MacLeod
1981	Alexander Albert
1982	Eugenia Rosemberg
1983	Kristen B.D. Eik-Nes
1984	Mortimer B. Lipsett
1985	Robert H. Foote
1986	Alfred D. Jost
1987	Emil Steinberger
1988	Yves W. Clermont
1989	C. Alvin Paulsen
1990	Marie-Claire Orgebin-Crist
1991	Philip Troen
1992	C. Wayne Bardin
1993	Anna Steinberger
1994	Richard J. Sherins
1995	Rupert P. Amann
1996	J. Michael Bedford
1997	Brian P. Setchell
1998	Ryuzo Yanagimachi
1999	Richard D. Amelar
2000	Bayard T. Storey
2001	Frank S. French
2002	Geoffrey M. H. Waites
2003	David M. de Kretser
2004	Ronald Swerdloff
2005	Mitch Eddy
2006	Norman Hecht
2007	Eberhard (Ebo) Nieschlag
2008	Bernard Robaire
2009	William Bremner
2010	Dolores Lamb
2011	Barry Zirkin

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

distinguished service award

Terry R. Brown, PhD

Terry R. Brown, PhD, is this year's recipient of the American Society of Andrology Distinguished Service Award. Dr. Brown is currently on faculty at both the Johns Hopkins Bloomberg School of Public Health and the Johns Hopkins University School of Medicine, where he is a professor in the department of biochemistry and molecular Biology, division of reproductive biology, as well as the department of pediatrics, division of endocrinology.

Terry is one of a special group of ASA members who have served the society in many different roles, demonstrating a wide range of skills as well as exemplary dedication. The Distinguished Service Award requires that the recipient has "served the society in at least three of the following categories: (1) Chair of Standing Committee; (2) Member of Executive Council; (3) Editor of *Journal of Andrology*; (4) Chair of Annual Meeting; (5) Officer of Society." Terry began his work for the society by serving as chair of the Constitution and Bylaws Committee in 1987, and then on the editorial board of the *Journal of Andrology* for 1990–1992. He also served on the Executive Council for a three-year term starting in 1991. Since that fast start, his service has been unbroken. He has had three more terms on the Executive Council over the last fifteen years, another term on the *Journal of Andrology* editorial board from 2008–2011, and has served on multiple committees as well. Terry has also been chair of several committees including the Constitution and Bylaws Committee, the Nominating Committee (twice), the Local Arrangements Committee (1997), and the Program Committee (2000). These chair positions are all important jobs for the society, but Terry has also taken on the heavy lifting by serving the society in all four officer positions: as treasurer (1996–1999) and secretary (2001–2004) before being elected vice-president (2006–2007) and president (2007–2008). Aside from those official duties, Terry has been a consistent, active participant in ASA annual meetings both as a presenter of brief reports in platform and poster sessions and as a main speaker.

Terry's tenure as president was noted for its quality leadership, almost unprecedented organization, as well as innovations that benefited the society. Most notably, Terry started the tradition of having two program chairs, one representing the clinical side, and one representing the basic side. The 2000 ASA Annual Meeting program, chaired by Terry, was strong evidence of his work ethic and the breadth of his knowledge, as the program was far-reaching and forward-thinking. Among the scientific topics were cutting-edge tissue engineering work, a symposium on cell fate decisions (an extremely hot area even now, 11 years later), and a discussion on gender assignment and gender identity.

Aside from his work in direct service to the society, Terry brings great credit to the organization by being a well-known, respected scientist and long-time contributor to the scientific literature on the molecular basis of androgen action and the endocrinology and molecular and cell biology of the prostate. The Distinguished Service Award to Terry is richly deserved due to his exemplary service and dedication to the ASA.

Distinguished Service Award Recipients

1994	C. Alvin Paulsen
1995	Andrzej Bartke
1996	Philip Troen
1997	Marie-Claire Orgebin-Crist
1998	Rupert P. Amann
1999	David W. Hamilton
2000	Bernard Robaire
2001	Gail S. Prins
2002	Terry T. Turner
2003	Arnold M. Belker
2004	J. Lisa Tenover
2005	Barry Hinton
2006	Barry Zirkin
2007	Sally Perreault Darney
2008	Matthew P. Hardy
2009	Erwin Goldberg
2010	Joel L. Marmar
2011	Christina Wang, MD

young andrologist award

Wei Yang, MD, PhD

Wei Yang, MD, PhD, is this year's recipient of the American Society of Andrology Young Andrologist Award. Dr. Yang is an associate professor in the department of physiology and cell biology at the University of Nevada School of Medicine in Reno, Nevada. He earned his MD degree in 1990 from China Medical University in Shenyang. From 1996–2000, Wei lived in Turku, Finland, and did his PhD studies on the expression, regulation, and function of stem cell factor in the rat testis under the supervision of Dr. Jorma Toppari at the University of Turku. Wei then pursued postdoctoral training at Baylor College of Medicine in Houston with Dr. Martin Matzuk. In 2004, he was appointed to his current position.

Wei has received numerous awards and honors including the Erns Schering Research Foundation Young Scientist Fellowship in 2002 and the Society for the Study of Reproduction Young Investigator Award in 2009. He is an active member of the Endocrine Society, the American Society of Andrology, and the Society for the Study of Reproduction. His current interests are in the regulation of protein turnover by ubiquitination during spermiogenesis, the control of cytoplasm removal during sperm individualization and spermiation, and the physiological roles of germ cell-specific small non-coding RNAs. His research has been supported by the NIH and by the Ernst Research Foundation. He has more than 60 papers in well recognized peer-reviewed journals and is recognized as a very talented mentor, having already supervised more than 30 students at the undergraduate, graduate and postgraduate levels.

Wei is building an outstanding scientific career and his colleagues recognize him as a talented scientist with innovative thinking. In summary, for his accomplishments and his scientific and mentor qualities, 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	1993	Robert Chapin	2004	Kate Loveland
1983	William B. Neaves	1994	Wayne J.G. Hellstrom	2005	Janice Bailey
1984	Lonnie D. Russell	1995	Christopher DeJonge	2006	Janice P. Evans
1985	Bruce D. Schanbacher	1996	Paul S. Cooke	2007	John K. Amory
1986	Stephen J. Winters	1997	Gail A. Cornwall	2008	Moira K. O'Bryan
1987	Ilpo T. Huhtaniemi	1998	William R. Kelce	2009	Michael A. Palladino
1988	Larry Johnson	1999	Stuart E. Ravnik	2010	Peter Liu
1989	Barry T. Hinton	2000	Matthew P. Hardy	2011	Humphrey Lao, PhD
1990	Luis Rodriguez/Rigau	2001	Jacquetta Trasler		
1991	Patricia M. Saling	2002	Christopher L.R. Barratt		
1992	Gary R. Klinefelter	2003	Joanna E. Ellington		

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 2012 Outstanding Trainee Investigator Award will be announced during the Annual Business Meeting on Monday, April 23, 2012 at 5:30 p.m.

New Investigator Award Recipients

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

Outstanding Trainee Investigator Award Recipients

2004	Darius Paduch
2005	Tara Barton
2006	Liwei Huang
2007	Steve Tardif
2008	Duangporn Jamsai
2009	Catherine Itman
2010	Michael Elliott
2011	Matthew Marcello

thanks to donors, supporters & exhibitors

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

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West Michigan Reproductive Institute
Texas Institute for Reproductive Medicine and Endocrinology

Silver Level
(Multiple or single contribution(s) greater than or equal to $5,000)
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Gail S. Prins, PhD
J. Lisa Tenover, MD, PhD
The Lalor Foundation, Inc.
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Susan Ann Rothmann, PhD, HCLD
Women in Andrology

Sustaining
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Dolores J. Lamb, PhD
Douglas T. Carrell, PhD
E. Mitch Eddy, PhD
Erwin Goldberg, PhD
Frank S. French, MD
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World Health Organization

Annual Contributions for 2010 (through 12/6/10)

$1000+
Douglas T. Carrell, PhD
Christina Wang, MD

$250-$999
Geng-Long Hsu, MD
Bayard T. Storey, PhD
J. Lisa Tenover, MD, PhD

$100-$249
Andrzej Bartke, PhD
Luiz Renato de Franca, PhD
Mary Ann Handel, PhD
Rex A. Hess, MS, PhD
Marvin L. Meistrich, PhD
Makoto Nagano, PhD, DVM
Douglas Stucco, PhD
Paul Jacob Turek, MD
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Barry R. Zirkin, PhD

$50-$99
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George L. Gerton, PhD
F. Kent Hamra, PhD
Carin V. Hopps, MD
Masaichi Kimura, MD, PhD
Clinton MacDonald, PhD
Antoine A. Makhlouf, MD, PhD
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course needs & objectives

37th Annual ASA Meeting
"Male Reproductive Health: This Generation to the Next"

Needs
Male reproductive function, fertility and sexual health are regulated by genetically and epigenetically programmed pathways that are initiated *in utero* and continue throughout adulthood. Research in humans and animal models indicates that these various pathways can be altered by environmental factors such as nutrition and toxicant exposure. Moreover, there is evidence that environmentally-mediated male reproductive dysfunction is transgenerational and paternally-mediated. Therefore, the modern clinical andrologist needs to integrate physiology, endocrinology, genetics, epigenetics, neurobiology, psychology and consideration of lifestyle and environmental exposures to provide optimal patient care. Adequately fostering such a systems approach to understanding normal reproductive function and related pathologies requires interaction between clinicians and translational scientists.

To address this objective, the 37th Annual Meeting of American Society of Andrology will permit clinicians and basic scientists to exchange ideas and raise new clinically applicable questions that can lead to novel fundamental research directions. Renowned researchers working in the fields of urology, endocrinology, clinical andrology, genetics, reproductive medicine and reproductive biology will present cutting edge developments in the physiological and molecular foundations of male reproductive function.

Learning Objectives
By the end of the ASA 37th Annual Meeting, attendees should be able to:
- Describe genetic and clinical aspects of the hypothalamic-pituitary axis and hypogonadism in male reproduction and infertility.
- Explain genetic, lifestyle, and environmental factors involved in the paternal contributions to embryo quality, reproductive success, and risk to the next generation.
- Identify the genetic foundations of male infertility.
- Describe current mechanisms of how the testicular environment supports male germ cell development and function.
- Describe state-of-the-art technologies and targets for male contraception and treatment of erectile dysfunction.
- Explain current mechanisms of androgen actions in target tissues and how androgen actions affect various male reproductive processes and pathologies, including spermatogenesis, infertility, and prostate cancer.
- Identify the effects of aging on prostate growth.

ASA Special Symposium

Needs
Sexual dysfunction affects roughly 52% of men and 43% of women over the age of 40. Although there are many etiologies for sexual dysfunction in both men and women, low testosterone is often an underlying cause in most instances. Roughly 40% of men over the age of 45 suffer from low testosterone and the prevalence of hypogonadism increases with age. The aim of this program is to provide attendees with a state-of-the-art overview of treating men and women with sexual dysfunction and low testosterone. We seek to educate clinicians on the more challenging issues such as testosterone replacement therapy after prostate cancer, testosterone's effect on cardiovascular disease, and treating sexual dysfunction in men with Peyronie's disease.

Educational Objectives:
By the end of the ASA Symposium, attendees should be able to:
- Review the controversy and challenges of treating men with testosterone after a history of prostate cancer.
- Identify male and female patients with hypogonadism and female sexual dysfunction and review treatment options.
- Describe current and novel therapies of testosterone replacement therapy.
- Explain the cardiovascular risks associated with testosterone replacement therapy.
- Identify and treat women with hypoactive sexual desire disorder.
- Identify the physiology and pathophysiology of erectile dysfunction and Peyronie's disease.
- Identify patients with Peyronie's disease and erectile dysfunction and list current treatments including penile prosthesis and grafts.

Accreditation Information
Visit the ASA website for accreditation updates:
www.andrologysociety.org

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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 38th Annual Conference and Testis Workshop
April 10 – 16, 2013
Hyatt Regency San Antonio
San Antonio, TX

Testis Workshop
April 10 – 13, 2013

Andrology Lab Workshop
April 13 – 14, 2013

ASA Special Symposium
April 13, 2013

schedule of events

Andrology Lab Workshop
"Sperm Morphology: Interactive Instruction of a New Rational Classification Method"
April 21 – 22, 2012
Hilton Tucson El Conquistador
Tucson, Arizona
Location: Joshua Tree
Program Chair: Dean Morbeck, PhD, HCLD

SATURDAY, APRIL 21, 2012

7:00 a.m. – 7:00 p.m. **Registration/Information Desk Open**
Location: Turquoise Foyer

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

8:30 a.m. – 9:30 a.m. **History of Sperm Morphology Classification Systems and Why They Have Failed; Introduction to Workshop Methods**
Susan Rothmann, PhD, HCLD
Fertility Solutions Inc.

9:30 a.m. – 10:00 a.m. **Classification Algorithm 101**
Anna Bort
Fertility Solutions Inc.

10:00 a.m. – 10:15 a.m. **Break—Visit Exhibits**

10:15 a.m. – 11:00 a.m. **Interactive Algorithm Instruction 1**
Anna Bort
Fertility Solutions Inc.
Aniela Bollendorf, MT
Cooper Institute for Reproductive Hormonal Disorders

11:00 a.m. – 11:20 a.m. **Practice with Virtual Smears**
Participants

11:20 a.m. – 11:45 a.m. **Interactive Review of Virtual Smears**
Anna Bort
Fertility Solutions Inc.
Aniela Bollendorf, MT
Cooper Institute for Reproductive Hormonal Disorders

11:45 a.m. – 1:15 p.m. **Lunch – Lab Science Forum**
Joseph Alukal, MD
NYU School of Medicine
Location: Agave

1:30 p.m. – 2:20 p.m. **Interactive Instruction 2**
Anna Bort
Fertility Solutions Inc.

2:20 p.m. – 2:50 p.m. **Practice with Virtual Smears**
Participants

2:50 p.m. – 3:00 p.m. **Break—Visit Exhibits**

3:00 p.m. – 3:30 p.m. **Interactive Review of Virtual Smears**
Anna Bort
Fertility Solutions Inc.
Aniela Bollendorf, MT
Cooper Institute for Reproductive Hormonal Disorders

3:30 p.m. – 4:30 p.m. **Reference Limits and Classification Problems**
Dean Morbeck, PhD, HCLD
Mayo Clinic

SUNDAY, APRIL 22, 2012

7:00 a.m. – 7:00 p.m. **Registration/Information Desk Open**
Location: Turquoise Foyer

7:00 a.m. – 8:00 a.m. **Continental Breakfast**

8:00 a.m. – 9:00 a.m. **Quality Control – Understanding QC Requirements and Construction & Use of Control Charts; Strategies to Prevent and Correct Classification Drift and Shift**
Susan Rothmann, PhD, HCLD
Fertility Solutions Inc.

9:00 a.m. – 9:30 a.m. **Distinguished Andrologist Award/ Break**

9:30 a.m. – 10:00 a.m. **Difficult Classifications**
Anna Bort
Fertility Solutions Inc.
Aniela Bollendorf, MT
Cooper Institute for Reproductive Hormonal Disorders

10:00 a.m. – 10:30 a.m. **Practice with Virtual Smears**
Participants

10:30 a.m. – 10:45 a.m. **Break – Visit Exhibits**

10:45 a.m. – 11:30 a.m. **Interactive Review of Difficult Classifications**
Anna Bort
Fertility Solutions Inc.

11:45 a.m. – 12:00 p.m. **Wrap-Up Discussion and Q&A**
Panel

schedule of events

Basic Science Workshop

"Diagnosing Male Reproductive Capacity in the Laboratory"

April 21, 2012
Hilton Tucson El Conquistador
Tucson, Arizona
Location: Coronado Ballroom
Program Chair: Kate Loveland

SATURDAY, APRIL 21, 2012

9:00 a.m. – 10:45 a.m. **Session 1: Histological Approaches**

How to Approach the Problem of Identifying Histopathological Changes in the Testis that Lead to Infertility, Including Cases that Illustrate Unique Approaches to the Challenges of Identifying STAGES
Rex A. Hess, PhD
University of Illinois, USA

10:45 a.m. – 11:15 a.m. **Break—Visit Exhibits**

11:15 a.m. – 1:00 p.m. **Session 2: Markers and Mechanisms**

Cell Fate and Transcriptional Outcomes Following Exposure to Endocrine Disruptors
Sara Pacheco and Ed Dere, PhD,
Brown University, USA (representing Boekelheide Lab)

Sperm Chromatin Structure Analysis
Cristian O'Flaherty, PhD
McGill University, Canada

1:00 p.m. – 1:45 p.m. **Lunch**

1:45 p.m. – 4:00 p.m. **Session 3: New Tools for Discovery**

Looking at Spermatogenesis from a Cellular Biophysicist's Perspective
Rich Cardullo, PhD
University of California, Riverside, USA

Dynamic Nuclear Substructure Analysis Using Confocal Microscopy and Imaris
Andy Major
Monash University, Melbourne, Australia

Bioinformatics of Spermatogenesis: A Biologist's Perspective
Liza O'Donnell, PhD
Prince Henry's Institute, Melbourne, Australia

ASA Special Symposium

"The Science of Couples Sexual Health"

Saturday, April 21, 2012
Hilton Tucson El Conquistador
Tucson, Arizona
Location: Turquoise I–II
Program Chairs: Allen D. Seftel, MD, FACS and Mohit Khera, MD

SATURDAY, APRIL 21, 2012

Testosterone and Prostate Cancer
Moderators: William J. Bremner, MD, PhD and Mohit Khera, MD

8:45 a.m. – 9:15 a.m. **Prostate Cancer and Testosterone – Time to Change**
Stephanie T. Page, MD, PhD

9:15 a.m. – 9:45 a.m. **Prostate Cancer and Testosterone – The Changing Paradigm**
Abraham Morgentaler, MD

9:45 a.m. – 10:00 a.m. **Questions and Answers**

10:00 a.m. – 10:15 a.m. **Break—Visit Exhibits**

Testosterone – New Horizons
Moderators: Allen D. Seftel, MD, FACS and Glenn R. Cunningham, MD

10:15 a.m. – 10:50 a.m. **Current Therapies**
Andre T. Guay, MD

10:50 a.m. – 11:30 a.m. **Novel Therapies**
Christina Wang, MD

11:30 a.m. – 12:10 p.m. **Testosterone and Cardio-Vascular Disease**
Robert Kloner, MD, PhD

12:10 p.m. – 12:30 p.m. **Questions and Answers**

12:30 p.m. – 1:15 p.m. **Lunch Lecture**
Update on T Assays
Shalender Bhasin, MD

Female Sexual Dysfunction
Moderators: Andre T. Guay, MD and Mohit Khera, MD

1:15 p.m. – 1:45 p.m. **Hypoactive Sexual Desire Disorder and Related Sexual Issues in Premenopausal Women**
Sharon Parish, MD

schedule of events

1:45 p.m. – 2:15 p.m. **Princeton III and the Heart in Female Sexual Dysfunction**
Martin Miner, MD

2:15 p.m. – 2:45 p.m. **Hormonal Replacement for Female Sexual Dysfunction**
John E. Buster, MD

2:45 p.m. – 3:15 p.m. **Questions and Answers**

3:15 p.m. – 3:25 a.m. **Break—Visit Exhibits**

Update on Erectile Dysfunction and Peyronie's
Moderators: Dana A. Ohl, MD and Mohit Khera, MD

3:25 p.m. – 3:50 p.m. **Erectile Dysfunction and Peyronie's Disease – Basic Science**
Tom F. Lue, MD

3:50 p.m. – 4:15 p.m. **Erectile Dysfunction and Peyronie's Disease – Treatment Options**
William O. Brant, MD

4:15 p.m. – 4:30 p.m. **Questions and Answers**

Epididymis Mini-Workshop

"The Epididymis in Infertility and as a Target for Contraceptive Development"

April 24, 2012
Immediately after the close of the 2012 ASA
Hilton Tucson El Conquistador
Tucson, Arizona
Location: Joshua Tree
Program Chairs: Terry T. Turner, PhD and Gail Cornwall, PhD

TUESDAY, APRIL 24, 2012

1:00 p.m. – 1:15 p.m. **Opening Remarks**
Terry T. Turner, PhD

1:15 p.m. – 1:40 p.m. **What Do We Know Now About Epididymal Immunobiology?**
Nicolas Da Silva, PhD

1:40 p.m. – 2:05 p.m. **What Do We Know About the Epididymal Luminal Protein Environment of the Epididymis?**
Gail A. Cornwall, PhD

2:05 p.m. – 2:30 p.m. **What Do We Know About Sperm Membrane Modifications in the Epididymis that are Associated with Gain or Loss of Function?**
Kenneth P. Roberts, PhD

2:30 p.m. – 2:55 p.m. **The Blood-Epididymis Barrier: A Hurdle or a Gateway for Male Contraceptive Development?**
Barry T. Hinton, PhD

2:55 p.m. – 3:20 p.m. **What Do We Know About Regulatory Relationships Between Cells in the Epididymal Epithelium and Between Cells of the Epithelium and the Cell Populations of the Epididymal Interstitium?**
Sylvie Breton, PhD

3:20 p.m. – 3:50 p.m. **Break**

3:50 p.m. – 4:20 p.m. **What Do We Know About the Role of the Epididymis in Male Infertility?**
Robert E. Brannigan, MD

4:20 p.m. – 4:45 p.m. **Crossing the Divide: Major Issues and Difficulties in Moving an Epididymal Target from a Discovery in the Laboratory to a Useful Product in the Clinic – One Investigator's Experience**
Michael G. O'Rand

4:45 p.m. – 5:05 p.m. **Male Reproductive Health Branch: What the Male Reproductive Health Program Within the NICHD is Interested in Regarding Male Infertility and What Assets We can Bring to Bear**
Stuart B. Moss, PhD

5:05 p.m. – 5:30 p.m. **Contraception Branch: What the Contraceptive and Reproductive Health Branch Within NICHD is Interested in Related to Male Contraceptive Development and What Assets We can Bring to Bear**
Diana Blithe, PhD

5:30 p.m. **Closing**
Gail A. Cornwall, PhD

schedule of events

ASA 37th Annual Meeting
"Male Reproductive Health: This Generation to the Next"
April 21 – 24, 2012
Hilton Tucson El Conquistador
Tucson, Arizona
Location: Turquoise I-III
Program Chairs: Janice P. Evans, PhD and Ajay K. Nangia, MBBS

FRIDAY, APRIL 20, 2012

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

SATURDAY, APRIL 21, 2012

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

10:00 a.m. – 4:30 p.m. **Exhibit Hall Open**
Location: Turquoise III

7:30 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Turquoise III

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

6:10 p.m. – 6:30 p.m. **Updates from NICHD & NIEHS**
Stuart B. Moss, PhD
NICHD
Jerry J. Heindel, PhD
NIEHS

6:30 p.m. – 6:50 p.m. **Distinguished Andrologist Award**

6:50 p.m. – 7:50 p.m. **EMIL STEINBERGER MEMORIAL LECTURE**
Bedside to Bench: Discovery of Genes that Control Reproduction in Men
William Crowley, MD
Harvard Medical School
(Introduced by: Gail A. Cornwall, PhD)

7:50 p.m. – 9:30 p.m. **Welcome Reception**
Location: Turquoise III

SUNDAY, APRIL 22, 2012

6:30 a.m. – 8:00 a.m. **Past President's Breakfast**
Location: Copper Cactus Room

7:00 a.m. – 6:00 p.m. **Registration**
Location: Turquoise Foyer

7:00 a.m. – 4:00 p.m. **Exhibit Hall Open**
Location: Turquoise III

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
Androgen Receptor Regulation in Prostate Cancer and Other Cells
Donald J. Tindall, PhD
Mayo Clinic
(Introduced by: Paul J. Turek, MD)

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

9:15 a.m. – 10:45 a.m. **SYMPOSIUM I**
Paternal Contributions to Embryo Quality and Reproductive Success
Session Chairs:
Sarah Kimmins, PhD
Mike Eisenberg, MD

Father Knows Best: Paternal Impacts of Embryogenesis
Douglas T. Carrell, PhD
University of Utah

Paternal Reprogramming of Metabolic Gene Expression in Offspring
Oliver Rando, MD, PhD
University of Massachusetts

Effects of First- and Second-Hand Tobacco Smoke: How Does It Affect Male Reproductive Function
Francesco Marchetti, PhD
Health Canada, Ottawa

10:45 a.m. – 11:00 a.m. **Quick Break**

11:00 a.m. – 12:30 p.m. **Poster Session I**
Location: Exhibit Hall

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; tickets required)
Location: Presidio I
"My First 'Real' Job: What is It Like to Be an 'Early Stage' Professional?"
Speakers: Distinguished Panel

12:30 p.m. – 2:00 p.m. **Editorial Board Luncheon**
Location: Agave I, II

schedule of events

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

Oral Session I: New Discoveries on Therapeutic and Diagnostic Tools for Male Reproductive Health

Location: Turquoise I - II

Moderators:
Hari K. Koul, PhD
James F. Smith, MD

2:00 p.m. – Abstract #1

IMPROVEMENTS OF COMPONENTS OF THE METABOLIC SYNDROME UNDER TESTOSTERONE TREATMENT OVER 48 MONTHS IN THREE COHORTS, IN TOTAL, 410 MEN

Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Aksam Yassin, MD, PhD[3], Michael Zitzmann, MD, PhD[4], Louis Gooren, MD, PhD[5] and Eberhard Nieschlag, MD, PhD[4]

[1]Scientific Affairs Men's Healthcare, Bayer Pharma AG; [2]Private Urology Practice, Bremerhaven, Germany; [3]Segeberger Kliniken, Norderstedt, Germany; [4]University of Muenster, Muenster, Germany; [5]VUMC Amsterdam, Amsterdam, The Netherlands

(Presented by: Farid Saad, DVM, PhD)

2:15 p.m. – Abstract #2

SERUM INSL3 CONCENTRATION IS HIGHLY CORRELATED WITH INTRATESTICULAR TESTOSTERONE CONCENTRATION IN NORMAL MEN STIMULATED BY VERY LOW DOSE HUMAN CHORIONIC GONADOTROPIN DURING EXPERIMENTAL GONADOTROPIN DEFICIENCY

Mara Roth, MD[1], Kat Lin, MD[1], Katrine Bay, MSc, PhD[2], John Amory, MD, MPH[1], Bradley Anawalt, MD[1], Alvin Matsumoto, MD[3], Brett Marck, BS[3], William Bremner, MD, PhD[1] and Stephanie Page, MD, PhD[1]

[1]University of Washington; [2]Rigshospitalet; [3]Veterans Affairs Puget Sound Health Care System

(Presented by: Mara Roth, MD)

2:30 p.m. – Abstract #3

LONG-TERM SURVIVAL AND DEVELOPMENT OF XENOGRAFTED HUMAN FETAL TESTICULAR TISSUE IN IMMUNODEFICIENT MICE

Yingchun Zhu[1], Kirk Lo, MD[2], Brendan Mullen, MD[2], Keith Jarvi, MD[2] and Colin McKerlie, DVM, PhD[3]

[1]Mount Sinai Hospital; [2]Mount Sinai Hospital, Toronto; [3]Hospital for Sick Children, Toronto

(Presented by: Yingchun Zhu, MS)

2:45 p.m. – Abstract #4

3D IMAGE ANALYSIS OF CHROMATIN STRUCTURE OF MOTILE AND IMMOTILE SPERM POPULATIONS

Naazish Alladin, BSc[1,2], Shlomit Kenigsberg, PhD[1], Clifford Librach, MD[1,3] and Sergey Moskovtsev, MD, PhD[1,4]

[1]CReATe Fertility Centre; [2]Department of Biomedical Sciences, Eastern Virginia Medical School, Norfolk; [3]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics & Gynaecology, Sunnybrook Health Sciences Centre; [4]Department of Obstetrics & Gynaecology, University of Toronto

(Presented by: Naazish Alladin, BSc)

3:00 p.m. – Abstract #5

VALPROIC ACID REVERSES PENILE FIBROSIS AND ERECTILE DYSFUNCTION IN CAVERNOUS NERVE INJURY RATS

Johanna Hannan, PhD, Omer Kutlu, MD, Xiaopu Liu, BS, Sena Sezen, PhD, Arthur Burnett, MD, MBA and Trinity Bivalacqua, MD, PhD

Johns Hopkins Medical Institutes

(Presented by: Johanna Hannan, PhD)

6:15 p.m. – Abstract #6

MOLECULAR INTERACTIONS OF GALECTIN-3 AND PSA IN HUMAN SEMEN, PROSTATE, AND PROSTATE TUMORS

Alan Diekman, PhD[1], Matthew Kovak, BS, MS[2], Sabrina Goddard[2], Rebecca Gilbride, BS[2], Ashley Block, BS, MS[2] and Sarika Saraswati, PhD[2]

[1]Department of Biochemistry and Molecular Biology; [2]UAMS

(Presented by: Alan Diekman, PhD)

2:00 p.m. – 3:30 p.m. **Oral Session II: Male Fertility—Spermatogenesis, Tract Biology and Sperm Function**

Location: Coronado Ballroom

Moderators:
William Wright, PhD
Thomas Garcia, PhD

2:00 p.m. – Abstract #7

A SPERM STEM CELL BASED FORWARD GENETIC SCREEN FOR REPRODUCTION PHENOTYPES IN THE LABORATORY RAT

Gerardo A. Medrano, BS[1,2], Jaideep Chaudhary, BS[1,2], Heather M. Powell, BS[1,2], Karen M., Chapman BS[1,2] and F. Kent Hamra, PhD[1,2]

[1]Department of Pharmacology; [2]Cecil H. & Ida Green Center for Reproductive Biology Sciences, UT Southwestern Medical Center

(Presented by: F. Kent Hamra PhD)

2:15 p.m. – Abstract #8

CONSTITUTIVE EXPRESSION OF ACTIVATED NOTCH1 IN SERTOLI CELLS LEADS TO A LOSS OF GDNF AND CYP26B1 EXPRESSION, AND PREMATURE GONOCYTE DIFFERENTIATION

Thomas Garcia, PhD and Marie-Claude Hofmann, PhD

Department of Comparative Biosciences, University of Illinois at Urbana-Champaign

(Presented by: Thomas Garcia, PhD)

2:30 p.m. – Abstract #9

HIDDEN MESSAGES: SPERM MRNA TRANSCRIPTS AS BIOMARKERS OF LOW DOSE TESTICULAR INJURY IN THE RAT

Sara Pacheco, BS, Linnea Anderson, BS, MS, Moses Sandrof, BS, Susan Hall, BS and Kim Boekelheide, MD, PhD

Brown University

(Presented by: Sara Pacheco, BS)

2:45 p.m. – Abstract #10

REAL-TIME IN VIVO IMAGING OF ENDOCYTOSIS AND MICROVILLI EXTENSION IN EPIDIDYMAL CLEAR CELLS BY MULTIPHOTON INTRAVITAL MICROSCOPY

Ye Chun Ruan, PhD, Jeremy Roy, PhD and Sylvie Breton, PhD

Massachusetts General Hospital, Harvard Medical School, Program in Membrane Biology, Center for Systems Biology

(Presented by: Ye Chun Ruan, PhD)

3:00 p.m. – Abstract #11

CYTOPLASMIC DROPLETS FUNCTION AS AN ENERGY SOURCE ESSENTIAL FOR NORMAL SPERM EPIDIDYMAL MATURATION

Shuiqiao Yuan, MSc[1], Hui Xu, MD[2], Zhihong Zheng, MD, PhD[2] and Wei Yan, MD, PhD[1]

[1]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV, USA; [2]Department of Laboratory Animal Medicine, China Medical University, Shenyang, China

(Presented by: Wei Yan, MD, PhD)

schedule of events

3:15 p.m. – Abstract #12
JUNCTIONAL ADHESION MOLECULE-A (JAM-A) INTERACTS WITH CALCIUM/CALMODULIN-DEPENDENT SERINE KINASE (CASK) AND IS SERINE-PHOSPHORYLATED IN HUMAN AND MOUSE SPERM; ITS POTENTIAL ROLE IN ASTHENOZOOSPERMIA AND MALE SUBFERTILITY
Rolands Aravindan, PhD and Patricia Martin-DeLeon, PhD
University of Delaware
(Presented by: Patricia Martin-DeLeon, PhD)

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Exhibit Hall

4:00 p.m. – 4:45 p.m. **LECTURE I**
Hormone Replacement Therapy in Men of Reproductive Age
Edward D. Kim, MD
University of Tennessee
(Introduced by: Mark Sigman, MD)

4:45 p.m. – 5:30 p.m. **LECTURE II**
Crosstalk Between Sertoli and Germ Cells – How Does This Lead to Testicular Cell Differentiation?
Leslie L. Heckert, PhD
Kansas University Medical Center
(Introduced by: Kate Loveland, 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: Sunset Point – Outdoor Area
(Rain Location: Presidio I)

MONDAY, APRIL 23, 2012

7:00 a.m. – 6:00 p.m. **Registration**
Location: Turquoise Foyer

7:00 a.m. – 3:30 p.m. **Exhibit Hall Open**
Location: Turquoise III

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Paternal Obesity Affects Offspring Diabetes Risk
Margaret Morris, PhD
University of New South Wales, Australia
(Introduced by: Patricia Martin-DeLeon, PhD)

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

9:15 a.m. – 10:45 a.m. **SYMPOSIUM II**
Future Technologies and Targets for Improving Men's Health
Session Chairs:
Mohit Khera, MD
Polina Lishko, PhD

H2-Gamendazole, A New Non-Hormonal Oral Male Contraceptive: The Road to Clinical Trials
Joseph S. Tash, PhD
Kansas University Medical Center

Gene Therapy, Stem Cells, and Tissue Regeneration for Erectile Dysfunction: Past, Present, and Future
Trinity J. Bivalacqua, MD, PhD
Johns Hopkins University

Cavernous Nerve Regeneration as an ED Therapy
Carol A. Podlasek, PhD
Northwestern University

10:45 a.m. – 11:00 a.m. **Quick Break**

11:00 a.m. – 12:30 p.m. **Poster Session II**
Location: Exhibit Hall

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

12:30 p.m. – 2:00 p.m. **WOMEN IN ANDROLOGY LUNCHEON AND DISCUSSION**
(Not included in registration fee; tickets required)
Location: Agave

2:00 p.m. – 3:30 p.m. **SYMPOSIUM III**
Male Germ Cells – From Their Birth to Their Grave
Session Chairs:
Marie-Claude Hofman, PhD
Andrew Major, BSci

Human Deleted in AZoospermia (DAZ) Gene Family – 600 Million Years in the Making
Eugene Xu, PhD
Nanjing Medical University

The Rules of Transcriptional Regulation of Spermatogenesis: Keep It Simple
Prabhakara Reddi, PhD
University of Virginia

schedule of events

Sertoli Cells: Not Just Nurse Cells But Undertakers Too
Jeffrey J. Lysiak, PhD
University of Virginia

3:30 p.m. – 4:00 p.m. **Refreshment Break**
Location: Exhibit Hall

4:00 p.m. – 4:45 p.m. **LECTURE III**
How a Sperm Learns to Be Fertile
Harvey M. Florman, PhD
University of Massachusetts Medical School
(Introduced by: Michael K. Holland, PhD)

4:45 p.m. – 5:30 p.m. **LECTURE IV**
Aging and Benign Prostatic Hyperplasia – What's the Connection?
Jill A. Macoska, PhD
University of Michigan
(Introduced by: Terry R. Brown, PhD)

5:30 p.m. **ASA Business Meeting Outstanding Trainee Investigator and Trainee Awards**

7:00 p.m. – 11:00 p.m. **Annual Banquet**
(Not included in registration fee; tickets required)
Location: The Last Territory, Hilton Tucson El Conquistador

TUESDAY, APRIL 24, 2012

7:00 a.m. – 8:00 a.m. **2013 Program Committee Meeting**
Location: Boardroom

7:30 a.m. – 12:15 p.m. **Registration**
Location: Turquoise Foyer

8:00 a.m. – 9:30 a.m. **SYMPOSIUM IV**
Androgen Actions and Responsive Tissues
Session Chairs:
Jacques Tremblay, PhD
Johanna Hannan, PhD

Cross Talk of the Androgen Receptor and DNA Damage Pathways: Molecular and Translational Prostate Cancer Relevance
Karen E. Knudsen, PhD
Thomas Jefferson University

Applying "-omics" Technology to Understand Sperm Production
Liza O'Donnell, PhD
Prince Henry's Institute

Dissecting Androgen Action: New Clues from Conditional Knockout Mice
Lee B. Smith, PhD
MRC, Human Reproductive Sciences Unit

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

9:45 a.m. – 10:45 a.m. **INTERNATIONAL LECTURE**
Environmental Perturbations and Vulnerabilities in Male Reproductive Health
Jorma Toppari, MD, PhD
University of Turku, Finland
(Introduced by: Patricia S. Cuasnicu, PhD)

10:45 a.m. – 12:15 p.m. **SYMPOSIUM V:**
Genetic Foundations of Male Infertility
Session Chairs:
Kirk C. Lo, MD
Sherwin Zagaroff, MD

Reproductive Fitness of the Human Y Chromosome
Sjoerd Repping, PhD
University of Amsterdam

New Insights in Genetics of Oligozoospermia in Infertile Men
Alexander Yatsenko, MD, PhD
University of Pittsburgh

Mutations in X-Linked Genes as Cause of Infertility in Men
Jeremy Wang, MD, PhD
University of Pennsylvania

MEETING ADJOURNED

Disclaimer Statement
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speaker abstracts

SATURDAY, APRIL 21, 2012
6:50 p.m. – 7:50 p.m.

EMIL STEINBERGER MEMORIAL LECTURE

BEDSIDE TO BENCH: DISCOVERY OF GENES THAT CONTROL REPRODUCTION IN MEN

William F. Crowley Jr., MD
Harvard Medical School

In the 40 years since the discovery of GnRH in 1971, the biology of the neurons that control its secretion and thereby human reproduction has unfolded slowly. Initially this quest for an improved understanding of the underlying biology of GnRH and its application to clinical medicine was predominantly guided by reductionist systems using basic biochemistry, pharmacology, animal studies, cell lines, and in vitro molecular approaches. Beginning in 1989, however, the availability of the sequence of the human genome enabled entirely new and more powerful insights into this area predominantly via the careful phenotypic and genetic study of various human models in which gene mutations alter the normal physiology of GnRH secretion and hence the timing, onset, or pace of sexual development. Foremost among these conditions are men and women with Kallmann Syndrome and normosmic Idiopathic Hypogonadotropic Hypogonadism, both of which result in an isolated defect in GnRH secretion.

Using these human models, 15 new genes crucial for normal functioning of the GnRH neuronal network have been discovered. Moreover, they have assembled themselves into 3 different groups according to their presumed site of action: a) neurodevelopmental genes (e.g. KAL1, NELF, FGF8, FGFR1); b) neuroendocrine genes (GnRH, GnRHR, TAC3, TAC3R, KISS1, KISS1R); and c) those 'overlap' genes that have clinical features of both groups (CHD7, FGFR1, PROK2, PROKR2).

This Keynote Lecture will provide some perspective on this human model and the genetic insights into the CNS control of reproduction provided by these human models. It will also demonstrate how these genes not only account for rare Mendelian disorders but also contribute to the more common reproductive failures.

SUNDAY, APRIL 22, 2012
8:00 a.m. – 9:00 a.m.

AUA LECTURE

ANDROGEN RECEPTOR REGULATION IN PROSTATE CANCER AND OTHER CELLS

Donald J. Tindall, PhD
Professor of Urology, Mayo Clinic, Rochester, MN

Objectives: The androgen receptor (AR) signaling axis plays a critical role in the development, function and homeostasis of the prostate. The classical action of AR is to regulate gene transcriptional processes via AR nuclear translocation, binding to androgen response elements on target genes and recruitment of, or crosstalk with, transcription factors. Prostate cancer initiation and progression is also uniquely dependent on AR. Androgen deprivation therapy remains the standard of care for treatment of advanced prostate cancer. Despite an initial favorable response, almost all patients invariably progress to a more aggressive, castrate-resistant phenotype. Considerable evidence now supports the concept that development of castrate-resistant prostate cancer (CRPC) is causally related to continued transactivation of AR. Understanding the critical events and complexities of AR signaling in the progression to CRPC is essential in developing successful future therapies. The purpose of my research is to understand the mechanisms by which the AR regulates this androgen refractory phenotype.

Methods: Cell lines and xenograft models that recapitulate the clinical phenotype of CRPC were used to study the role of the AR in CRPC. shRNA was used to knock down expression of the AR and expression constructs were used to enhance expression of AR.

Results: We have discovered novel AR Variants that are expressed in CRPC cells. Most of these AR Variants do not contain the Ligand-Binding Domain of the protein. Functionally, these AR Variants are constitutively active and promote the expression of endogenous AR-dependent genes, as well as the proliferation of CRPC cells in a ligand-independent manner. AR mRNAs containing cryptic exons and their protein products are expressed in commonly studied PCa cell lines, xenograft-based animal models, as well as prostate tumors from the clinic. Some AR Variants appear to have prognostic utility.

Conclusions: Together, our data describe a simple and effective mechanism by which PCa cells can synthesize a constitutively active AR and thus circumvent androgen ablation.

Funding: Funding provided by NIH grants CA121277 and CA91956 and The T.J. Martell Foundation.

SUNDAY, APRIL 22, 2012
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Paternal Contributions to Embryo Quality and Reproductive Success

FATHER KNOWS BEST: PATERNAL IMPACTS ON EMBRYOGENESIS

Douglas T. Carrell, PhD, HCLD
Division of Urology, University of Utah School of Medicine

Objectives: Traditionally, the sperm cell has been viewed as a transport vehicle of twenty-three chromosomes to the oocyte during fertilization. While the unique and elegant specialization of the sperm cell in delivering the paternal genome has been appreciated, the paternal genome has been viewed as a rather static contribution to the embryo, with little practical contribution to early embryogenesis. The objective of this presentation is to review recent sperm epigenetic studies detailing the sperm methylome and chromatin modifications in the context of their likely role in early embryogenesis.

Methods: Sperm chromatin dynamics will be reviewed.

Results: Most canonical histones are replaced during spermiogenesis with protamines, which are central to silencing the sperm DNA, packing sperm chromatin, and protecting the DNA from damage. Retained histones are not randomly dispersed throughout the genome, rather are enriched in the gene promoters of developmental genes, micro RNAs, and imprinted genes. Many developmentally relevant gene promoters have a bivalent enrichment of histones (H3K4me3/H3K27me3) similar to stem cells. Additionally the promoters are demethylated. Interestingly, limited genome-wide studies of some infertility patients, including men with poor embryogenesis during IVF, have demonstrated altered histone enrichment and aberrant DNA methylation.

Conclusions: These early studies demonstrate that sperm epigenetic marks likely portend a functional role of the sperm genome in guiding transcription during embryogenesis.

speaker abstracts

SUNDAY, APRIL 22, 2012
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Paternal Contributions to Embryo Quality and Reproductive Success

PATERNAL REPROGRAMMING OF METABOLIC GENE EXPRESSION IN OFFSPRINg

Oliver Rando, MD, PhD
Biochemistry and Molecular Pharmacology, University of Massachusetts

SUNDAY, APRIL 22, 2012
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Paternal Contributions to Embryo Quality and Reproductive Success

EFFECTS OF FIRST- AND SECOND-HAND TOBACCO SMOKE: HOW DOES IT AFFECT MALE REPRODUCTIVE FUNCTION?

Francesco Marchetti, PhD, ScD and Carole L. Yauk, PhD
Health Canada, Ottawa, ON, Canada
(Presented by: Francesco Marchetti)

Objectives: Substantial evidence from human studies is associating paternal cigarette smoking with increased chromosomal abnormalities in sperm and increased risk for spontaneous abortions, birth defects and neonatal death. However, the mechanisms by which paternal smoking may damage germ cells and harm the developing embryo remain to be elucidated. We used a mouse model to determine whether exposure to first- and second-hand smoke affects the function and integrity of mouse sperm, and to determine whether the effects differ between the two types of smoke.
Methods: Male mice were exposed to two doses of mainstream tobacco smoke (MTS), the smoke inhaled by active smokers, or sidestream smoke (STS), the main component of second-hand smoke. Animals were exposed in inhalation chambers to 3 or 16 cigarettes per day for two weeks using a cigarette smoking machine, alongside sham controls. Sperm function and integrity measures included: motility, DNA damage, and DNA mutations. Analyses conducted at different times from the end of the exposure were utilized to assess the effects of tobacco smoking on specific stages of mouse spermatogenesis. Exposure to tobacco smoke was assessed by measuring cotinine levels in plasma.
Results: Based on cotinine levels, exposures were comparable to light smokers (<5 cigarettes/day) and passive smokers for MTS and STS, respectively. Specific differences in sensitivity were noted for germ cells following MTS and STS exposure including: (1) only STS reduced sperm motility; (2) only MTS smoke induced DNA strand breaks in sperm; (3) both MTS and STS smoke increased sperm chromatin structure abnormalities; and (4) both MTS and STS smoke caused DNA mutations in sperm. Interestingly, for the majority of the endpoints investigated, there was little evidence for dose-related effects, as exposure to low doses of MTS and STS were as effective as high doses. Furthermore, the detrimental effects on motility and sperm DNA integrity persisted for several weeks after the end of exposure, indicating that differentiating spermatogonia are also susceptible to tobacco smoke.
Conclusions: These results show that MTS and STS smoke have differential effects on the genetic integrity and function of sperm. Importantly, the results show that both MTS and STS can induce mutations in sperm that can be passed on to future generations with potentially detrimental effects on their health. These data provide further evidence that male exposure to second-hand smoke, as well as direct cigarette smoke, is likely to have reproductive consequences that go beyond the passive smoker.
Funding: Funding provided by the California Tobacco Related Disease Research Program (grant 13RT-0140A) and the Canadian Regulatory Systems for Biotechnology.

SUNDAY, APRIL 22, 2012
4:00 p.m. – 4:45 p.m.

LECTURE I

HORMONE REPLACEMENT THERAPY IN MEN OF REPRODUCTIVE AGE

Edward D. Kim, M.D
University of Tennessee Graduate School of Medicine, Knoxville, TN

Needs Assessment Statement:
Testosterone therapies have been increasingly utilized in aging men, as well as in men of reproductive age. With the recent introduction of several newer commercial testosterone preparations and an increased public awareness of androgen deficiency syndromes, use of hormone replacement therapies (HRT) is likely to grow. However, men desiring to maintain their reproductive potential may not be fully aware of the risks of exogenous testosterone therapy. Unfortunately, many physicians are not aware that HRT may suppress spermatogenesis. In a recent study surveying physicians practice patterns, it was identified that approximately 30% of physicians have used exogenous testosterone to treat low testosterone levels associated with male infertility. At present, there is a distinct lack of published recommendations or guidelines on this topic.

Learning Objectives:
At the conclusion of this session, the participant should be able to:

- Estimate the number of men who may be affected by hypogonadism within the reproductive years, the increasing age of paternity, and the number of young men being treated for hypogonadism.
- Identify the mechanisms by which testosterone replacement therapy inhibits spermatogenesis.
- Discuss the potential for reversibility of spermatogenesis and its timecourse after HRT.
- Recognize the importance of intrinsic testosterone for spermatogenesis and how intrinsic testosterone can be maintained.
- Recommend therapeutic strategies including use of hCG and selective estrogen modulators (SERMs).

SUNDAY, APRIL 22, 2012
4:45 p.m. – 5:30 p.m.

LECTURE II

CROSSTALK BETWEEN SERTOLI AND GERM CELLS—HOW DOES THIS LEAD TO TESITICULAR CELL DIFFERENTIATION?

Valentine Agbor[1], Tatiana Karpova[1], Daren A. Rice[1], Ning Lei[2], Tao Shixin[1], and Leslie L. Heckert[1]
[1]Department of Molecular and Integrative Physiology University of Kansas Medical Center, Kansas City, KS; [2]The Simons Center for Systems Biology, School of Natural Sciences, Institute for Advanced Study, Princeton, NJ
(Presented by: Leslie L. Heckert)

Objectives: The transcription factor DMRT1 (Doublesex and Mab-3 related transcription factor 1) is uniquely expressed in spermatogonia and Sertoli cells, where it is an essential for spermatogenesis. To enhance our knowledge of male fertility and the mechanistic requirements for spermatogenesis, studies were undertaken to determine how DMRT1 is restricted to Sertoli cells and germ cells and what specific functions it performs within them.
Methods: To determine how *Dmrt1* is directed to Sertoli cells and germ cells, 5kb of 5' flanking sequence was examined for elements and proteins

that regulate *Dmrt1* transcriptional activity. Transient transfections, transgenic mice, and electrophoretic mobility shift assays, combined with mutagenesis, were used to identify DNA sequences and transcription factors directing testis expression. To ascribe DMRT1 functions to Sertoli cells and germ cells, *Dmrt1*$^{-/-}$ mice and Sertoli cell-expressing DMRT1 transgenic mice were characterized for morphological, molecular, and functional changes in the testis. Direct and indirect targets of DMRT1 activity were identified by chromatin immunoprecipitation followed by deep sequencing (ChIP-SEQ) and microarray analysis, respectively. Functional associations were drawn from genes differentially expressed between *Dmrt1*$^{+/+}$ and *Dmrt1*$^{-/-}$ testes and those located near confirmed DMRT1 binding sites.
Results: Two major regulatory regions were identified within the *Dmrt1* 5' flanking region. The proximal region directed transgenic reporters to the testis but not to Sertoli cells and was largely controlled by elements bound by SP1/3 and EGR transcription factors. The distal region was required for Sertoli cell-specific expression, as shown by transient transfection and transgenic mouse studies. Key regulatory elements in this region bound GATA4 and were inhibited by FOXL2, a transcription factor needed for ovarian differentiation. ChIP-SEQ identified many novel binding sites and associated DMRT1 target genes. The target genes fit into functional groups having obvious biological ties to the differentially expressed testis genes. The data suggests a model whereby DMRT1 controls genes in Sertoli cells and germ cells that are important to their direct interaction and communication.
Funding: U54 HD055763 Interdisciplinary Center for Male Contraceptive Research and Drug Development (Joseph Tash, PI).

MONDAY, APRIL 23, 2012
8:00 a.m. – 9:00 a.m.

WOMEN IN ANDROLOGY LECTURE

PATERNAL OBESITY AFFECTS OFFSPRING DIABETES RISK

Margaret J. Morris, Sheau-Fang Ng
Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia
(Presented by: Margaret J. Morris)

Objectives: Increased childhood obesity is associated with the early emergence of metabolic disease across the globe. Having either parent obese is an independent risk factor for childhood obesity. Our laboratory is examining the contribution of parental obesity to offspring outcomes in the rat. Both genetic and environmental factors are recognized as important contributors to maternal transmission of obesity. The role of the father's environment in transmitting obesity to his offspring has been less well explored.
Methods: Male Sprague Dawley rats were fed high fat chow or control chow for 12 weeks before mating with weight-matched females consuming control chow. Chronic high fat diet consumption in Sprague Dawley fathers induced increased body weight, adiposity, impaired glucose tolerance and insulin sensitivity. Female offspring were weaned at 3 weeks onto control chow. Glucose tolerance was performed at 6 and 12 weeks of age and islets were harvested at 14 weeks.
Results: The female offspring of obese fathers had no evidence of altered adiposity at 14 weeks, but showed reduced glucose tolerance and insulin secretion from 6 weeks of age. Underlying mechanisms included reduced β-cell reserve due to a lack of large-sized islets and altered islet gene expression, including genes involved in calcium and insulin signalling, Wnt signalling and the MAPK pathway. The islet gene with the greatest alteration in expression, interleukin 13 receptor a2 (Il13ra2), showed reduced DNA methylation, providing evidence of epigenetic alteration.
Conclusions: These findings extend the concept of developmental and adaptive plasticity to include a paternal role in the early life origins of disease. The critical window for the developing germ cell to be vulnerable to environmental insult remains to be elucidated. Further studies are needed to examine potential consequences of paternal consumption of high fat diet, including intergenerational transmission.

MONDAY, APRIL 23, 2012
9:15 a.m. – 10:45 a.m.

SYMPOSIUM II – Future Technologies and Targets for Improving Men's Health

H2-GAMENDAZOLE, A NEW NON-HORMONAL ORAL MALE CONTRACEPTIVE: THE ROAD TO CLINICAL TRIALS

Joseph S. Tash
Interdisciplinary Center for Male Contraceptive Research & Drug Development, University of Kansas Medical Center, Kansas City, KS; Department of Molecular & Integrative Physiology, Department of Urology, University of Kansas Medical Center, Kansas City, KS

Objectives: Previous studies reported on a gamendazole (GMZ), a non-hormonal contraceptive agent that, at the lowest single oral dose that gave 100% infertility in rats, showed 60% recovery of fertility. In order to develop a viable reversible contraceptive drug candidate, the goal was to use a new analogue, H2-GMZ, to establish anti-spermatogenic efficacy in multiple mammalian species, and to show improved contraceptive reversibility.
Methods and Results: Single oral doses of H2-GMZ showed significant dose-related reductions in spermatid counts in mouse, rat, and rabbit. Pilot studies in non-human primates (Rhesus) showed significant and reversible reductions in semen sperm count and spermatogenic index at single oral doses from 0.6 - 2.0mg/kg, with no adverse side effects at any of these doses. H2-GMZ showed 100% oral bioavailability in rats, and excellent liver metabolic stability, as well as Ames and hERG tests negative. Dose finding studies of H2-GMZ in rats showed that by significantly lowering the amount per dose at repeated intervals, selective loss of spermatids with excellent retention of spermatogonia and spermatocytes was achieved. Mating trials using single consecutive weekly oral doses of H2-GMZ at 2mg/kg/dose and 1mg/kg/dose per wk achieved 100% and 95% infertility, respectively. Full recovery of fertility was achieved 8-10wks after the last dose in both treatment groups. Early studies using affinity pull-down suggested that HSP90 and eEF1A were direct binding targets of H2-GMZ. Isothermal calorimetry has ruled out HSP90 as a direct binder; yet, HSP pathways are transiently upregulated by H2-GMZ. H2-GMZ binding to eEF1A was confirmed and specifically inhibited the non-canonical F-actin bundling activity without affecting the nucleotide binding nor ribosome charging/peptide elongation eEF1A functions. In addition, uptake of H2-GMZ by Sertoli cells was more rapid than all other cells tested so far.
Conclusions: Our current model indicates that H2-GMZ's contraceptive effect is based on transient disruption of the apical ectoplasmic specializations (aES) that tether spermatids to Sertoli cells by causing structural disruption of the aES through unbundling of the associated F-actin filaments. H2-GMZ shows particular promise as a reversible non-hormonal male contraceptive agent. The combined efficacy and safety data have allowed a pre-IND package to be prepared to approach the FDA to enable H2-GMZ as an IND to move forward towards first in human clinical trials.
Funding: Supported by NIH U54 HD055763 to JST.

speaker abstracts

MONDAY, APRIL 23, 2012
9:15 a.m. – 10:45 a.m.

SYMPOSIUM II – Future Technologies and Targets for Improving Men's Health

GENE THERAPY, STEM CELLS AND TISSUE REGENERATION FOR ERECTILE DYSFUNCTION: PAST, PRESENT AND FUTURE

Trinity J. Bivalacqua, MD
Assistant Professor of Urology and Oncology, Johns Hopkins Medical Institutions

Erectile dysfunction (ED) is a major health problem that seriously affects the quality-of-life of patients and their partners. ED is mainly associated with vascular disease including diabetes mellitus, hypertension, hyperlipidemia, cardiovascular diseases, and smoking, and its prevalence significantly increases with aging. Although all three selective type 5 phosphodiesterase (PDE5) inhibitors are effective in the majority of ED cases, PDE5 inhibitor therapy is less efficacious in some hard-to-treat populations (diabetics, post-radical prostatectomy). Only about 50% to 65% of these cases benefit from PDE5 inhibitor therapy, prompting the development of new approaches, including gene and stem cell-based therapy strategies for ED. The penis is a convenient tissue target for wither gene or stem cell based therapies because of its external location and accessibility, the ubiquity of endothelial lined spaces, rich smooth muscle cell content, and neuronal innervations as well as low level of blood flow, especially in the flaccid state. Gene therapy approaches have focused on a number of signaling pathways that are crucial for penile erection, such as nitric oxide/cyclic guanosine monophosphate, RhoA/Rho-kinase, growth factors, ion channels, peptides, and controlling oxidative stress. Various viral and nonviral vectors have been used to date for the transfer of genetic material to target cells or tissues with various degrees of success. With the advent of adult stem cell therapies for regeneration of new healthy tissue, in particular neuronal, endothelial and smooth muscle cells, the use of this form of therapy may be utilized to improve erectile function in the penile vascular bed. Gene modified ex vivo expanded adult stem cells are attractive for use in stem cell based therapy since they avoid some of the risks and disadvantages associated with direct in vivo delivery of viral vectors, non-viral vectors, or gene modified ex vivo expanded differentiated cells. Another approach which has been used in other genitourinary diseases is a regenerative medicine approach to restoration of whole organ function and replacement. Over the last decade, we have learned a great deal from gene and stem cell based therapies in pre-clinical animal models as well as the first erectile dysfunction gene therapy clinic trial in men with severe ED. In this presentation, I will review lessons learned and the future of ED therapies with a particular emphasis on gene and stem cell based therapies.

MONDAY, APRIL 23, 2012
9:15 a.m. – 10:45 a.m.

SYMPOSIUM II – Future Technologies and Targets for Improving Men's Health

CAVERNOUS NERVE REGENERATION AS AN ED THERAPY

Carol A. Podlasek, PhD
Research Assistant Professor, Northwestern University

Objectives: The cavernous nerve (CN), which provides innervation to the penis, frequently undergoes resection, crush and tension injury during prostatectomy surgery, resulting in erectile dysfunction (ED). Penile tissues innervated by the damaged CN undergo smooth muscle apoptosis and fibrosis, resulting in significantly reduced effectiveness of PDE-5 inhibitors. Although peripheral nerves have a limited ability to regenerate, a return of function typically does not occur due to irreversible down stream morphological changes in the penis. Thus new therapies that address both the down stream changes in the penis and injury to the CN are necessary. The secreted protein sonic hedgehog (SHH) plays a prominent role in nerve development, and has been implicated as a critical factor in regeneration of peripheral nerves. We propose that SHH is essential to maintain CN integrity, and that local SHH protein treatment of the CN at the time of injury using novel peptide amphiphile (PA) nanofibers, will speed CN regeneration. We have examined this hypothesis in a Sprague Dawley bilateral CN crush rat model.
Methods: The localization of SHH pathway members was examined in normal pelvic ganglia (PG)/CN. The time course of SHH signaling after CN crush in the CN was determined by western analysis. Normal CNs were treated with 5E1 SHH inhibitor and CN morphology was examined by electron microscopy (EM). Anterograde and retrograde transport were inhibited and SHH signaling was examined in the CN. Rats underwent bilateral CN crush and CNs were treated with SHH or BSA by PA nanofibers. Intracavernosal pressure/blood pressure, EM, immunohistochemical analysis and *in situ* hybridization were performed. SHH protein was labeled with Alexa Fluor 488 and was given via PA to crushed CNs *in vivo*. *In vitro* release kinetics of SHH PA was examined.
Results: The SHH signaling pathway is localized in an intriguing pattern in neurons of the PG that innervate the penis. SHH protein is significantly decreased in the CN after crush injury. SHH inhibited CN showed axonal degeneration and demyelination. PA nanofibers were effective in delivering SHH to the PG/CN and SHH treatment is both neuroprotective in the crushed CN and promotes regeneration and return of erectile function. Signals from the PG and penis are required to maintain SHH in the CN.
Conclusions These findings suggest that there is a window of opportunity immediately after nerve insult in which manipulation of the nerve microenvironment can affect long-term regeneration outcome.
Funding: Funding provided by NIH/NIDDK DK079184

MONDAY, APRIL 23, 2012
2:00 p.m. – 3:30 p.m.

SYMPOSIUM III – Male Germ Cells—From Their Birth to Their Grave

HUMAN DELETED IN AZOOSPERMIA (DAZ) GENE FAMILY—600 MILLION YEARS IN THE MAKING

Eugene Yujun Xu
State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China; Department of Obstetrics and Gynecology, and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

Objectives: Our goal is to trace the origin and evolutionary history of DAZ gene family and to determine the relationship between DAZ family members and their homologs in other species.
Methods: We performed phylogenetic analyses, expression analyses involving RT-PCT, Real-time PCR and immunohistochemistry as well as functional analyses involving gene targeting technology and developmental biology and genetics methodology.
Results: DAZL homologs are found in mammals, birds, reptiles, amphibians and bony fish but absent in cartilaginous fish or other chordates or invertebrates, suggesting that Dazl arose after the split of bony fish and cartilaginous fish. On the other hand, BOULE homologs are present in the genomes of representative species of each of the major lineages of metazoans and exhibit reproductive-specific expression in all species examined, with a preponderance of male-biased expression. Furthermore, loss of function of mammalian Boule resulted in male-specific infertility and a global arrest of sperm development remarkably similar to the phenotype in an insect boule mutation.

Conclusions: This work traces the origin of human DAZ gene family to the dawn of animal evolution when ancient Boule arose 600 million years ago. Hence Boule is the oldest member of the DAZ family and gave rise to Dazl during vertebrate evolution and DAZ arose during recent primate evolution.
We also demonstrated the conservation of a reproductive protein throughout eumetazoa, its predominant testis-biased expression in diverse bilaterian species, and conservation of a male gametogenic requirement in mice. This shows an ancient gametogenesis requirement for Boule among Bilateria and supports a model of a common origin of spermatogenesis.
Funding: Provided by NIH NCHD and Nanjing Medical University Talent Fund.

MONDAY, APRIL 23, 2012
2:00 p.m. – 3:30 p.m.

SYMPOSIUM III – Male Germ Cells—From Their Birth to Their Grave
THE RULES OF TRANSCRIPTIONAL REGULATION OF SPERMATOGENESIS: KEEP IT SIMPLE
Prabhakara Reddi, PhD
Department of Pathology, University of Virginia Medical School

In this theme, the differentiation of spermatogonial stem cells into mature spermatozoa may be considered as the youth to grave phase. Men produce approximately 1000 sperm per second from age 12 onwards. Proper execution of a number of molecular events is critical for keeping spermatogenesis on track throughout life. The purpose of this presentation is to review the transcriptional regulatory strategies which ensure that spermatogenic differentiation markers are expressed in a precise spatiotemporal order during male germ cell differentiation.
Gene knockouts in mice showed that transcription factors PLZF, A-myb, and CREM-tau (expressed in spermatogonia, spermatocytes, and spermatids, respectively) are essential for spermatogenesis, indicating control by master transcription factors. Promoter analysis at the level of candidate genes led to the remarkable finding that the proximal promoter regions (approximately 200 bp) are sufficient for spermatogenic cell-specific transcription in vivo. Interestingly, the same promoter can also prevent transcription in the somatic tissues by acting as a repressor or insulator. This is in complete contrast to the typical arrangement of enhancers and repressors several kilobases away from the proximal promoters (e.g., immunoglobulin genes). In addition to the testis/somatic ON/OFF mechanism, fine-tuning of gene expression during spermatogenesis itself is brought about by RNA Pol II pausing. Our work using the mouse acrv1 gene as a model showed that although the acrv1 mRNA is expressed in round spermatids, the proximal promoter becomes poised for transcription in spermatocytes. Chromatin IP showed the presence of bivalent histone marks as well as phosphorylation marks typical of paused and elongating RNA Pol II in spermatocytes and spermatids, respectively. Again, the cis-elements critical for this mechanism reside within the proximal promoter.
Thus, male germ cell-specific transcriptional regulation is kept simple by the alternate usage of the proximal promoter as an enhancer, repressor, or insulator. The mechanisms involved will be the focus of future studies.
Funding: R03HD062710

MONDAY, APRIL 23, 2012
2:00 p.m. – 3:30 p.m.

SYMPOSIUM III – Male Germ Cells—From Their Birth to Their Grave
SERTOLI CELLS: NOT JUST NURSE CELLS BUT UNDERTAKERS TOO
Jun Zhang[1], Deaho Park[3], Kodi S. Ravichandran[2,3] and Jeffrey J. Lysiak[1]
Departments of Urology[1], Microbiology[2], and the Beirne B. Carter Center for Immunology Research[3], University of Virginia, Charlottesville, VA
(Presented by: Jeffrey J. Lysiak, PhD)

Each day billions of cells in our bodies die via apoptosis, and efficient clearance of these apoptotic cell corpses is essential for normal tissue homeostasis. Recently we reported that clearance of apoptotic germ cell corpses by Sertoli cells is a fundamental part of spermatogenesis and revealed a role for the engulfment protein ELMO1. ELMO1, together with DOCK1, function as a guanine nucleotide exchange factor for the small GTPase RAC1 leading to reorganization of the cytoskeleton during engulfment of apoptotic cells. We now define the role of RAC1 in Sertoli cell mediated clearance of apoptotic germ cells and provide evidence of how failed corpse clearance disrupts spermatogenesis. Mice expressing Cre under control of the *anti-mullerian hormone* promoter (*Amh*-Cre) were crossed with floxed *Rac1* transgenic mice to generate Sertoli cell-specific *Rac1* deficient mice (*Amh*-Cre+/*Rac1*^{F,F}). *Amh*-Cre^{+}/*Rac1*$^{F/F}$ mice were viable and grew to adulthood; however, adult male mice had an 80% reduction in testes size and a severely disrupted seminiferous epithelium. siRNA knockdown of *Rac1* in Sertoli cells significantly inhibited engulfment of surrogate apoptotic targets. Engulfment assays using primary Sertoli cells isolated from *Amh*-Cre+/*Rac1*^{F,F} mice showed a significant reduction in the internalization of apoptotic germ cells. To investigate how failed germ cell corpse clearance disrupts spermatogenesis we examined the blood testis barrier (BTB). Immunostaining for ZO-1 and occludin, markers of Sertoli cell tight junctions, as well as endogenous albumin revealed random staining for ZO-1 and occludin with intraluminal albumin staining in the seminiferous tubules suggesting a breakdown of the BTB. Intratesticular microinjection of a peptide that blocks the recognition of apoptotic cells by Sertoli cells, also lead to failed removal of apoptotic germ cells and disruption of the BTB. Our results demonstrate that RAC1 is an essential member of the Sertoli cell engulfment module necessary for the clearance of apoptotic germ cells and that failed clearance of apoptotic germ cells leads to breakdown of the BTB, resulting in disruption seminiferous epithelium homeostasis and ultimately loss of spermatogenesis.

MONDAY, APRIL 23, 2012
4:00 p.m. – 4:45 p.m.

LECTURE III
HOW A SPERM LEARNS TO BE FERTILE
Harvey M. Florman, Melissa K. Jungnickel, Keith A. Sutton, Wenlei Cao, Tsai Pei-Shiue. Department of Cell Biology, University of Massachusetts Medical School, Worcester MA
(Presented by: Harvey M. Florman)

Mammalian sperm, including those of humans, are infertile when released by the male and acquire the ability to fertilize eggs during residence within the female reproductive tract. This functional reprogramming, or capacitation, has been studied extensively in vitro. In contrast, little is known about the mechanisms that control the onset of sperm fertility in vivo. We have used a variety of genetic models to study these events as they occur in the oviduct.

speaker abstracts

MONDAY, APRIL 23, 2012
4:45 p.m. – 5:30 p.m.

LECTURE IV
AGING AND BENIGN PROSTATIC HYPERPLASIA—WHAT'S THE CONNECTION?
Jill A. Macoska, PhD
The University of Michigan

Benign prostatic hyperplasia (BPH), or non-cancerous enlargement of the prostate, is a common condition associated with aging in men and is often synonymous with lower urinary tract symptoms (LUTS).
Studies from our laboratory have shown that a variety of chemokines consistent with a senescence-associated proteome are actively secreted by the prostatic microenvironment consequent to the aging process. Chronic prostatitis/ chronic pelvic pain syndrome (CP/CPPS) and histological inflammation may also serve as rich sources of chemokine secretion in the prostate and predispose to the later development of BPH and associated LUTS. Chemokines can stimulate powerful pro-proliferation signal transduction pathways and thus function as potent growth factors in the aging prostate, driving epithelial, mesenchymal, and endothelial proliferation associated with BPH and LUTS. Engineered over-expression of the murine form of the CXCL8 chemokine, KC, in the mouse prostate promotes myofibroblast accumulation, and human BPH tissues exhibit myofibroblast accumulation and concurrent over-expression of CXCL8. CXCL8 and other CXC-type chemokines also promote the accumulation of myofibroblasts and extracellular matrix components that alter prostate tissue architecture and promote urethral dysfunction contributing to LUTS. Finally, progressive declines in the production and tissue levels of the major prostate growth factor, testosterone, with age, may actually exacerbate TGFbeta- and chemokine-mediated changes in prostate tissue proliferation and architecture associated with LUTS.
Though the accumulated evidence is far from complete and suffers from some rather extensive gaps in knowledge, it argues favorably for the conclusion that chemokines can, and likely do, promote BPH/LUTS development and progression.
Funding: Supported by NIH/NIDDK grant 1P20DK090870-02 (JAM).

TUESDAY, APRIL 24, 2012
8:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Androgen Actions and Responsive Tissues
ANDROGEN AXIS DISRUPTION IN PROSTATE CANCER
Matthew J. Schiewer[1,6], Jonathan F. Goodwin[1,6], J. Chad Brenner[7,8,9], Michael A. Augello[1,6], Fengzhi Liu[2,6], Jamie L. Planck[3,6], Randy S. Schrecengost[1,6], Felix Y. Feng[7,10,13], Arul M. Chinnaiyan[7,8,9,11,12,13], Adam P. Dicker[1,5,6], Jonathan R. Brody[2,6], John M. Pascal[3,6], Karen E. Knudsen [1,4,5,6]
[1]Departments of Cancer Biology, [2]Surgery, [3]Biochemistry and Molecular Biology, [4]Urology, [5]Radiation Oncology and, [6]Kimmel Cancer Center Thomas Jefferson University, Philadelphia, PA, [7]Michigan Center for Translational Pathology, [8]Department of Pathology, [9]Program in Cellular and Molecular Biology, [10]Department of Radiation Oncology, [11]Howard Hughes Medical Institute, [12]Department of Urology, [13]Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI
(Presented by: Karen E. Knudson)

Prostate cancers (PCa) are exquisitely dependent on the action of the androgen receptor (**AR**) for cell survival and proliferation, and there is a significant need to develop new means for targeting recurrent AR activity in both locally advanced and castration-resistant PCa(1, 2). PARP1 (Poly ADP-ribose polymerase 1) is an enzyme that modifies a subset of nuclear proteins by poly (ADP-ribose)-ylation, and is known to play a critical role in base excision repair(3). This function of PARP1 has been cultivated as a therapeutic target for tumors tumors that harbor alterations of specific DNA repair pathways(4, 5). Multiple enzymatic inhibitors of PARP1 function are in clinical trial; while little dose limiting toxicity has been observed, suppressing PARP1-mediated DNA damage repair in BRCA1/2 deficient tumors leads to synthetic lethality and heightened clinical response to chemotherapy. Recently, it has been revealed that PARP1 has a second major cellular function on chromatin as a transcriptional coregulator, capable of modulating chromatin structure and selected transcription factor activity(6-8).

New observations in our laboratory point toward PARP1 inhibitors as a means to simultaneously dampen AR activity and sensitize PCa cells to genotoxic insult. This premise is based on three major arms of investigation. *First*, abrogation of PARP1 activity results in sensitization of both androgen deprivation-therapy (**ADT**) naïve and castration-resistant PCa cells to ionizing radiation, thus indicating that PARP1 activity plays a significant role in the cellular response to radiotherapy. *Second*, PARP1 activity was found to be increased as a function of tumor progression in model systems of human disease, suggesting that gain of PARP1 activity may promote resistance to combined ADT and radiotherapy. *Third*, robust molecular analyses indicate that PARP1 is recruited to sites of AR activity on chromatin, and therein serves as a requisite cofactor for AR activity. The dependence of AR on PARP1 activity is conserved in cells that failed hormone therapy, thus indicating that the requirement for PARP1 is maintained or enhanced during the process of tumor progression. Together, these data strongly support a model wherein the *dual functions of PARP1 in controlling AR activity and the response to radiotherapy can be leveraged to improve treatment of locally advanced prostate cancer.*

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TUESDAY, APRIL 24, 2012
8:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Androgen Actions and Responsive Tissues
APPLYING "-OMICS" TECHNOLOGY TO UNDERSTAND SPERM PRODUCTION
Liza O'Donnell
Prince Henry's Institute of Medical Research, Australia

Androgens are essential for sperm production. Data from transgenic mice suggest that androgens act directly on Sertoli cells, rather than germ cells,

to support spermatogenesis. Androgen action on Sertoli cells is necessary for the survival of pachytene spermatocytes and their final meiotic division, the elongation of haploid spermatids and their final release from Sertoli cells. How androgens regulate these processes is not well understood, and thus these studies sought to elucidate the genes, proteins and cellular processes that respond to androgens in Sertoli cells and germ cells. Transcriptional profiling of the androgen-responsive stages of spermatogenesis (VII and VIII in rats) revealed that androgens act on these stages to promote the down-regulation of specific cohorts of genes expressed by Sertoli cells, spermatogonia, and round spermatids in the preceding stages, and to coordinate the expression of cohorts of genes in Sertoli cells and spermatogonia needed in stage VII and beyond. Proteomic studies on the same tissues revealed changes in specific proteins, but none of these were changed at the mRNA level, suggesting that androgens also regulate translation. Arrays for microRNA (miRNA) species were then applied to these tissues, and showed that androgens control the expression of specific miRNAs in Sertoli cells which in turn modulate the translation of target proteins, such as those involved in endocytosis. Since the completion of meiosis is dependent on androgen action on Sertoli cells, meiotic cells were isolated after androgen suppression and/or replacement *in vivo* and the proteomic changes analysed. The data revealed that androgen, via Sertoli cells, regulates translation and post-translational modifications of proteins involved in the response to oxidative stress, cell survival/apoptosis, DNA repair and cellular division. In conclusion, data from these "omics" technologies reveal that androgens regulate Sertoli cell mRNA and miRNA transcription and protein translation and processing to control specific cellular responses. As yet unknown androgen-dependent stimuli from Sertoli cells in turn act on germ cell transcription and translation to modulate the completion of meiosis and spermiogenesis.

TUESDAY, APRIL 24, 2012
8:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Androgen Actions and Responsive Tissues

DISSECTING ANDROGEN ACTION: NEW CLUES FROM CONDITIONAL KNOCKOUT MICE

Lee Smith, PhD
MRC Centre for Reproductive Health, University of Edinburgh, UK.

Objectives: In males androgens are primarily made by testicular Leydig cells and act as essential regulators of both fetal masculinization and adult reproductive function. The impact of androgens on gene transcription is largely mediated by the Androgen Receptor (AR), a member of the steroid hormone super-family of ligand activated transcription factors. AR is expressed widely throughout the body, including several key somatic cell-types in the testis. Although we have known for many years that androgens are important regulators of testicular development and function, until recently it has been impossible to determine the specific roles androgens play in each cell-type, and how these cells respond to androgens to ensure correct male development and fertility.
Methods: We have exploited conditional gene-targeting of AR using the Cre/lox system to ablate AR function in several key cell-types of the testis, including the Sertoli cells (SC), Peritubular Myoid Cells (PTM), Vascular Smooth muscle cells (VSM), Vascular Endothelial cells (VE), and Leydig cells (LC); with a view to elucidating the cell-specific roles of androgen-signaling within the testis.
Results: These studies have identified novel roles for each cell-type in the promotion of male reproductive function. AR-signaling in SCs controls post-meiotic germ cell development and LC number. AR-signaling in PTM cells controls all stages of GC development, SC function and LC differentiation. Whilst AR-signaling in VE cells appears dispensable for testicular function, AR-signaling in VSM cells controls testicular blood-flow and LC function. Recent unpublished data suggests AR-signaling in LCs is also important for testicular function, acting via a novel mechanism.
Conclusions: Taken together, these studies provide increasing evidence for the presence of a complex androgen-dependent paracrine signaling pathway within the testis, with each AR-expressing cell-type influencing others to ensure their correct development and function.
Funding: Funding Provided by the UK Medical Research Council, The British Heart Foundation, and Tenovus.

TUESDAY, APRIL 24, 2012
9:45 a.m. – 10:45 a.m.

INTERNATIONAL LECTURE

ENVIRONMENTAL PERTURBATIONS AND VULNERABILITIES IN MALE REPRODUCTIVE HEALTH

Jorma Toppari, MD, PhD
Departments of Paediatrics and Physiology, University of Turku, FI-20520 Turku, Finland

Development of male reproductive system is regulated by several hormones. Accurate timing of the regulatory functions is crucial for normal development and e.g. androgen action at a critical male programming window determines later differentiation of sexually dimorphic features, such as anogenital distance. Perturbation of the regulation at a vulnerable developmental window can therefore cause irreversible long-term effects. Fetal exposure of experimental animals
to antiandrogens and estrogens cause reproductive disorders, such as cryptorchidism, hypospadias, testicular dysgenesis and subfertility. Thus, these disorders can share etiology in development, and therefore they can be considered as signs of Testicular Dysgenesis Syndrome (TDS). The big question is whether these disorders in human are also caused by endocrine disrupters and whether they should alert us of harmful exposures. While we still do not know a firm answer to this question, we know that the incidence of testicular cancer has rapidly increased over two generations, and the birth rates of hypospadias and cryptorchidism are high. Furthermore, semen quality of young European men remains very poor at the moment. In Finland, semen quality has deteriorated during the last 15 years. We have analyzed the association of cryptorchidism with exposure to several endocrine disrupters. This kind of studies cannot prove any causality. However, we have found a weak positive association of cryptorchidism with exposure to chlorinated pesticides and polybrominated diphenyl ethers. It has become obvious that there is no individual compound that could be the sole cause of cryptorchidism, but rather a mixture of several chemicals can cause the effect in genetically susceptible individuals. Modern systems biological approaches are needed to deal with complex exposure scenarios and genetic variability. First attempts to such approaches have revealed new genes that are associated with TDS disorders.
Supported by the Academy of Finland, EU Fp7 Environment DEER, Sigrid Juselius Foundation, Turku University Hospital, and Danish Council for Strategic research.

speaker abstracts

TUESDAY, APRIL 24, 2012
10:45 a.m. – 12:15 p.m.

SYMPOSIUM V – Genetic Foundations of Male Infertility
REPRODUCTIVE FITNESS OF THE HUMAN Y CHROMOSOME
Sjoerd Repping, PhD
Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

The human Y chromosome harbors many genes that are expressed specifically or predominantly in the testis. The structure of modern day Y chromosomes is the result of rapid and extensive rearrangements over the course of evolution. One of the main driving forces behind these structural rearrangements is reproductive fitness where some chromosomes are more likely to be transmitted to the next generation than others due to their effect on semen quality. Clinically, these evolutionary processes are visible among men with reduced semen quality as recurrent Y-chromosome aberrations. To date multiple Y-chromosome aberrations have been detected among men with reduces semen quality, including deletions, duplication, and isodicentric Y chromosomes. This lecture will provide a state-of-the-art update on these aberrations, their molecular origin as well as their implications for current clinical practice.
Disclosure: Nothing to disclose.

TUESDAY, APRIL 24, 2012
10:45 a.m. – 12:15 p.m.

SYMPOSIUM V – Genetic Foundations of Male Infertility
NEW INSIGHTS IN GENETICS OF OLIGOZOOSPERMIA IN INFERTILE MEN
Alexander Yatsenko
Magee Womens Research Institute at University of Pittsburgh, PA

Objectives: Oligozoospermia is one of the most common semen deficiencies diagnosed in the male infertility clinic. However, very few genetic defects in humans have been identified to cause this condition. Moreover, no molecular genetics diagnostic tests are clinically available for patients with oligozoospermia. Based on animal and expression studies of oligozoospermia, we know that several molecular pathways are disturbed in post-meiotic spermatozoa. One of the disrupted pathways is protein ubiquitination and germ cell apoptosis. Two well described proteins in the pathway are the ubiquitin conjugating enzyme 2B, UBE2B, and kelch-like 10, KLHL10.

Recent reports demonstrated that the absence of *Ube2b* and *Klhl10* in male mice is responsible for impaired post-meiotic stages of spermatogenesis, leading to male infertility. Also, human studies of *UBE2B* showed significant association between the gene SNPs and oligozoospermia.
Methods: To examine an association between *UBE2B* gene and severe oligozoospermia ($\leq 10^7$ cell/ml) we performed mutation screening of the genes coding region in spermatozoal cDNA in 200 oligozoospermic patients and 293 normozoospermic controls. Also, we performed genetic studies of *KLHL10*, *MSY2* and *SPAG16* genes. To study the role of genomic imbalances in oligozoospermia we utilized a novel high-throughput microarray technology, comparative genomic hybridization (CGH).
Results: We identified *UBE2B* mutations in 5/200 (2.5%) oligozoospermic patients and none in the respective controls. Additional study of 96 patients with mild oligozoospermia and dbSNP database did not reveal *UBE2B* mutations. Based on extensive *KLHL10* study results we propose genotype-phenotype correlation of the gene mutations. *MSY2* and *SPAG16* studies are in progress. In our initial CGH study we perform genome-wide screening of 20 severe oligozoospermic patients. We are currently analyzing patients' genomic data using Genomic Workbench software.
Conclusions: Our results corroborate previous animal and human association studies and indicate that UBE2B has an important role in late stages of spermatogenesis affecting sperm density.
Funding: Funding was provided by the National Institutes of Health (K08HD058073) and MWRI startup funds.

TUESDAY, APRIL 24, 2012
10:45 a.m. – 12:15 p.m.

SYMPOSIUM V – Genetic Foundations of Male Infertility
MUTATIONS IN X-LINKED GENES AS CAUSE OF INFERTILITY IN MEN
P. Jeremy Wang, MD, PhD
Center for Germ Cell Research, Dept. of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Objectives: Infertility is a worldwide reproductive health problem affecting men and women about equally. Genetic studies in various model organisms have greatly increased our understanding of fertility. More than 600 mouse mutants have infertility as a major phenotype. Despite the rapid progress made in model organisms, little progress has been made in translating these findings into identifying genetic causes of infertility in humans. Our objective is to search for single genic mutations in idiopathic infertile men and to validate these mutations as a cause of infertility in humans using mouse models.
Methods: The mammalian X chromosome is enriched for early spermatogenesis genes. Therefore, we hypothesize that X-linked germ cell-specific genes represent "hot spots" for infertility-causing mutations in men. As these are single-copy genes and males are hemizygous for the X chromosome, mutations in these X-linked genes, unlike autosomal recessive mutations, would not be masked by a wild type allele. We screened for mutations in two X-linked germ cell-specific genes (TEX11 and TAF7L) in 246 azoospermic men and 113 oligozoospermic men respectively by exome sequencing. We also screened 175 "control" men.
Results: We have identified a number of mutations in TEX11 and TAF7L in infertile men, including frameshift mutations, splice site mutations, missense mutations, etc. We have generated knockin mice to validate some missense mutations by modeling human male infertility in mouse.
Conclusions: We find that one missense mutation in TAF7L is likely to be the genetic cause of oligozoospermia in humans. Our data demonstrate that the infertility-causing mutation frequency of TEX11 in humans is at least 1% among azoospermic men. This finding is highly significant, given that at least several hundreds of genes specifically regulate fertility.
Funding: Funding provided by NIH/NICHD (R03 HD064628) and NIH/NIGMS (R01 GM076327).

poster session I

Sunday, April 22, 2012
11:00 a.m. – 12:30 p.m.
Location: Exhibit Hall

ANDROGENS / ENDOCRINOLOGY

Poster #13 **PENILE DYSMORPHOGENESIS IN RATS TREATED NEONATALLY WITH DIETHYLSTILBESTROL (DES) IS MEDIATED THROUGH STROMAL CELL REPROGRAMMING TOWARD INCREASED ADIPOGENESIS AND LOSS OF SMOOTH MUSCLE**

Lilian A. Okumu[1], Liz Simon[1], Tim Braden[2] and Hari Goyal[1]
[1]Tuskegee University; [2]Auburn University
(Presented by: Lilian A. Okumu)

Poster #14 **COMORBIDITIES IN MEN WITH "LATE ONSET HYPOGONADISM" TREATED IN TWO UROLOGICAL CENTRES OF COMPETENCE**

Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Aksam Yassin, MD, PhD[3] and Louis Gooren, MD, PhD[4]
[1]Scientific Affairs Men's Healthcare, Bayer Pharma AG; [2]Private Urology Practice, Bremerhaven, Germany; [3]Segeberger Kliniken, Norderstedt, Germany; [4]VUMC, Amsterdam, The Netherlands
(Presented by: Farid Saad, DVM, PhD)

Poster #15 **ANDROGENIC EFFECTS OF BASIL, OCIMUM BASILICUM, ON SPERMATOGENESIS DEFICIENCY PRODUCED BY EXPOSURE TO ELECTROMAGNETIC FIELD IN RATS.**

Arash Khaki, DVM, PhD[1], Fatemeh Fathi Azad, PhD[2], Mohamad Nouri, PhD[3] and Amir Afshin Khaki, PhD[4]
[1]Department of Veterinary Pathology, Tabriz Branch, Islamic Azad University, Tabriz-Iran; [2]Department of Pharmacognosy, Tabriz University of Medical Sciences; [3]Department of Biochemistry, Tabriz University of Medical Sciences; [4]Department of Anatomical Sciences, Tabriz University of Medical Sciences.
(Presented by: Arash Khaki DVM, PhD)

ENVIRONMENT / TOXICOLOGY

Poster #16 **HIDDEN MESSAGES: SPERM MRNA TRANSCRIPTS AS BIOMARKERS OF LOW DOSE TESTICULAR INJURY IN THE RAT**

Sara Pacheco, BS, Linnea Anderson, BS, MS, Moses Sandrof, BS, Susan Hall, BS and Kim Boekelheide, MD, PhD
Brown University
(Presented by: Sara Pacheco, BS)

Poster #17 **MEHP REDUCES MAINTENANCE AND PROLIFERATION OF PRIMARY SPERMATOGONIAL STEM CELLS IN VITRO**

Benjamin E. Lucas, MS and Marie-Claude Hofmann, PhD
University of Illinois – Comparative Bioscience and Institute for Genomic Biology
(Presented by: Benjamin E. Lucas, MS)

Poster #18 **THE IMPACT OF COMMERCIAL AND POTENTIAL PLASTICISERS ON HUMAN PROSTATE CELL LINE FUNCTIONS**

Claudia Lalancette, PhD[1] and Bernard Robaire, PhD[2]
[1]Pharmacology and Therapeutics, McGill University; [2]Departments of Pharmacology and Therapeutics and Obstetrics and Gynecology, McGill University
(Presented by: Claudia Lalancette, PhD)

EPIDIDYMIS / VAS DEFERENS / SEMINAL VESICLES

Poster #19 **TOXIC IMPACT OF ALUMINIUM—STUDY ON MEMBRANE BOUND ENZYMES AND SECRETORY PRODUCTS IN THE EPIDIDYMIS OF MATURE RATS**

Ramalingam Venugopal, PhD[1], Suganthy Onerine Marcelline, MSc, MPhil[2], Panneerdoss Subbarayalu, PhD[3] and Suryavathi Viswanadhapalli, PhD[3]
[1]Assistant Professor; [2]Research Scholar; [3]Post Doctoral Fellow
(Presented by: Ramalingam Venugopal, PhD)

poster session I

Poster #20 **NON CLASSICAL SECRETION BY PRINCIPAL CELLS OF THE EPIDIDYMIS**
Jennifer Hughes and Trish Berger, PhD
UCDavis
(Presented by: Jennifer Hughes)

Poster #21 **EXPRESSION OF THE MAJOR SPLICE VARIANTS OF PMCA4 IN THE MURINE EPIDIDYMIS AND CAUDAL SPERM: THE ROLE OF PMCA4A IN EPIDIDYMAL MATURATION AND SPERM FUNCTION**
Ramkrishna Patel, Undergrad[1], Deborah Stabley, BSc[2], Emanuel Strehler, PhD[3] and Patricia Martin-DeLeon, PhD[1]
[1]University of Delaware; [2]A.I. DuPont Hospital for Children; [3]Mayo Clinic College of Medicine
(Presented by: Patricia Martin-DeLeon, PhD)

FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT

Poster #22 **"POTENTIAL GENETIC BIOMARKERS IN AZOOSPERMIA BY MICROASSAY STUDIES: NEW DIMENSION IN THE EVALUATION"**
Vasan Satya Srini, DNB – Urology[1] and Srinivas Belur Veerachari, MBBS, MS[2]
[1]Medical Director – Ankur Health Care Private Limited, Director – Manipal Andrology and Reproductive Services; [2]Fellow Andrology – RGUHS
(Presented by: Vasan Satya Srini, DNB – Urology)

Poster #23 **SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN RAT SPERM CAPACITATION**
Maria Agustina Battistone, PhD student[1], Mariana Weigel Muñoz, PhD student[1], Juan I. Ernesto, PhD student[1], Julieta A. Maldera, PhD[1], Dario Krapf, PhD[2], Pablo Visconti, PhD[3] and Patricia S. Cuasnicu, PhD[1]
[1]IByME-CONICET; [2]IBR- CONICET; [3]University of Massachusetts
(Presented by: Maria Agustina Battistone, PhD student)

Poster #24 **GENETIC INFLUENCE ON CRISP1 KNOCK OUT MICE PHENOTYPE**
Mariana Weigel Muñoz, Maria Agustina Battistone, PhD student, Juan Ignacio Ernesto, PhD student, Gustavo Vasen, undergraduate, Vanina Gabriela Da Ros, PhD, Julieta Antonela Maldera, PhD, Debora Juana Cohen, PhD and Patricia S. Cuasnicu, PhD
IBYME-CONICET
(Presented by: Mariana Weigel Muñoz)

GENETICS

Poster #25 **SEMEN EXAMINATION AND CFTR GENE ANALYSIS IN PATIENTS WITH CYSTIC FIBROSIS AND CBAVD SYNDROME**
Vyacheslav Chernykh, Dr., Elena Amelina, Dr., Stanislav Krasovskiy, Dr., Tatiana Sorokina, Dr., Ludmila Shileiko, Dr., Lubov Kurilo, Prof., Nika Petrova, Dr., Anna Stepanova, Dr. and Aleksandr Polyakov, Prof., Dr.
(Presented by: Vyacheslav Chernykh, Dr.)

Poster #26 **CLINICALAND DIAGNOSTIC IMPLICATIONS OF LOW GRADE 46,XY/47,XXY MOSAICISM IN KLINEFELTER SYNDROME**
Akanksha Mehta, MD[1], Venkat R. Pulijaal, PhD[2], Susan Mathew, PhD[2], Carole Samang-Sprouse, EdD[3] and Darius A. Paduch, MD, PhD[1]
[1]Department of Urology, Weill-Cornell Medical College; [2]Department of Pathology and Laboratory Medicine, Weill-Cornell Medical College; [3]Department of Pediatrics, George Washington University
(Presented by: Akanksha Mehta, MD)

INFERTILITY / ART / MALE CONTRACEPTION

Poster #27 **CRISP1 IN SEMINAL PLASMA: AN ALTERNATIVE FOR THE DIFFERENTIAL DIAGNOSIS OF OBSTRUCTIVE AND NON-OBSTRUCTIVE AZOOPSERMIA**
Christine Legare, MSc, Francine Cloutier, Technician, Roland Tremblay, Physician and Robert Sullivan, PhD
Laval University
(Presented by: Christine Legare, MSc)

Poster #28 **SHORTENED SPERM TELOMERE LENGTH AND ITS ASSOCIATION WITH MALE INFERTILITY**
Thilagavathi Jayapalraja, MPharm, Rajeev Kumar, PhD and Rima Dada, MD, PhD
(Presented by: Thilagavathi Jayapalraja, MPharm)

Poster #29 **ANALYSIS OF GENETIC, MOLECULAR AND LIFE STYLE FACTORS IN IDIOPATHIC INFERTILE MEN**
Venkatesh Sundararajan, MPharm, PhD, Rajeev Kumar, MD and Rima Dada, MD, PhD
(Presented by: Venkatesh Sundararajan, MPharm, PhD)

Poster #30 **UTILIZATION OF FERTILITY PRESERVATION SERVICES IN NON-CANCER MEDICAL CONDITIONS**
Kunj Sheth, BS[1], Vidit Sharma, BA[1], Sherwin Zargaroff, BA, MD[2], Brian Le, MD[2] and Robert Brannigan, BA, MD[2]
[1]Feinberg School of Medicine Northwestern University; [2]Department of Urology, Feinberg School of Medicine Northwestern University
(Presented by: Brian Le, MD)

Poster #31 **RELATING ECONOMIC CONDITIONS TO VASECTOMY AND VASECTOMY REVERSAL RATES: A BI-INSTITUTIONAL PILOT STUDY**
Vidit Sharma, BA[1], Kunj Sheth, BS[1], Sherwin Zargaroff, MD[2], Brian Le, MD[3] and Robert Brannigan, MD[3]
[1]Feinberg School of Medicine; [2]Northwestern University Medical Center; [3]Department of Urology, Feinberg School of Medicine Northwestern University
(Presented by: Sherwin Zargaroff, MD)

Poster #32 **POST VASECTOMY REVERSAL SEMEN ANALYSIS COMPLIANCY**
Ryan Murphy, BS[1], Amy Perkins, MS[2], Matthew Marks, BS[2], Peter Burrows, MD[1] and Sheldon Marks, MD[1]
[1]International Center for Vasectomy Reversal; [2]Arizona Andrology Laboratory & Cryobank
(Presented by: Ryan Murphy, BS)

Poster #33 **BODY MASS INDEX DOES NOT PREDICT FOR INTRAOPERATIVE FINDINGS OR POST-OPERATIVE OUTCOMES WITH VASECTOMY REVERSAL**
Matthew Marks, BS[1], Amy Perkins, MS[1], Peter Burrows, MD[2] and Sheldon Marks, MD[2]
[1]Arizona Andrology Laboratory & Cryobank; [2]International Center for Vasectomy Reversal
(Presented by: Matthew Marks, BS)

MALE SEXUAL FUNCTION

Poster #34 **INCREASED SEXUAL DESIRE WITH EXOGENOUS TESTOSTERONE ADMINISTRATION IN MEN WITH OBSTRUCTIVE SLEEP APNEA: AN 18-WEEK RANDOMIZED DOUBLE-BLIND PLACEBO CONTROLLED STUDY**
Kerri Melehan, BCom, BAppSc, MAppSc, RPSGT[1], Camilla Hoyos, BSc (Hons), MPH[1], Brendon Yee, MBChB, FRACP, FCCP, PhD[2], Peter Buchanan, MBBS, MD, FRACP[3], Ron Grunstein, MBBS, MD, PhD[1] and Peter Liu, MBBS (Hons), PhD, FRACP[1]
[1]University of Sydney; [2]Royal Prince Alfred Hospital; [3]Liverpool Hospital
(Presented by: Peter Liu, MBBS (Hons), PhD, FRACP)

OTHER

Poster #35 **A NEW PROTEIN EXTRACTION PROTOCOL FOR THE PROTEOMIC STUDY OF TESTICULAR TISSUE**
Mohammed Mosli, MBBS[1], Ihor Batruch, MSc[2], Christopher Smith, MSc[2], Brendan Mullen, MD, FRCPC[3], Eleftherios Diamandis, MD, PhD, FRCP(C), FRSC[4] and Keith Jarvi, MD, FRCSC(C)[5]
[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada.; [2]Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada.; [3]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada. Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, Canada.; [4]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada. Department of Clinical Biochemistry, University Health Network, Toronto, ON, Canada.; [5]Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada. Department of Surgery (Division of Urology), Mount Sinai Hospital, Toronto, ON, Canada, Department of Surgery, University of Toronto, Toronto, Canada
(Presented by: Mohammed Mosli MBBS)

poster session I

PROSTATE / TESTIS CANCER / CLINICAL UROLOGY

Poster #36 **DOES ELEVATED BODY MASS INDEX (BMI) AFFECT THE CLINICAL OUTCOMES OF ROBOT-ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)?**

Xiao Gu, MD, PhD[1], Massimiliano Spaliviero, MD[2], David Turk, MD[3] and Carson Wong, MD[3]
[1]The First Clinical Medical College at Yangzhou University; [2]University of Oklahoma Health Sciences Center; [3]SouthWest Urology, Inc.
(Presented by: Carson Wong, MD)

Poster #37 **BLADDER NECK PRESERVATION WITH A RUNNING VESICOURETHRAL ANASTOMOSIS AND URINARY CONTINENCE FOLLOWING ROBOT-ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)**

Carson Wong, MD[1], Xiao Gu, MD, PhD[2], Massimiliano Spaliviero, MD[3] and David Turk, MD[1]
[1]SouthWest Urology, Inc.; [2]The First Clinical Medical College at Yangzhou University; [3]University of Oklahoma Health Sciences Center
(Presented by: Carson Wong, MD)

Poster #38 **BASELINE SEXUAL HEALTH INVENTORY FOR MEN (SHIM) PREDICTIVE OF ERECTILE FUNCTION FOLLOWING ROBOT-ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)**

Carson Wong, MD[1], Xiao Gu, MD, PhD[2], Massimiliano Spaliviero, MD[3] and David Turk, MD[1]
[1]SouthWest Urology, Inc.; [2]The First Clinical Medical College at Yangzhou University; [3]University of Oklahoma Health Sciences Center
(Presented by: Carson Wong, MD)

Poster #39 **DOES SIZE REALLY MATTER? THE IMPACT OF PROSTATE VOLUME ON THE EFFICACY AND SAFETY OF GREENLIGHT HPS™ LASER PHOTOSELECTIVE VAPORIZATION PROSTATECTOMY**

Carson Wong, MD[1], Xiao Gu, MD, PhD[2], Gino Vricella, MD[3] and Massimiliano Spaliviero, MD[4]
[1]SouthWest Urology, Inc.; [2]The First Clinical Medical College at Yangzhou University; [3]Case Western Reserve University; [4]University of Oklahoma Health Sciences Center
(Presented by: Carson Wong, MD)

SPERM FUNCTION / SEMEN ANALYSIS

Poster #40 **THERAPEUTIC EFFECT OF MUCUNA PRURIENS (LINN.) ON DIABETES INDUCED MITOCHONDRIAL DYSFUNCTION AND DNA DAMAGE IN RAT SPERM**

Seppan Prakash, PhD, Sekar Suresh, PhD, Elumalai Prithiviraj, MSc, Nagella Venkata Lakshmi, MSc, Mohanraj Karthik Ganesh, Msc and Lakshmanan Ganesh, MSc
University of Madras
(Presented by: Seppan Prakash, PhD)

Poster #41 **LC3B PROCESSING AND AN APOPTOSIS LIKE MECHANISM MAY HAVE A ROLE IN THE SURVIVAL OR DEATH OF STALLION SPERMATOZOA DURING CONSERVATION IN REFRIGERATION**

Juan Maria Gallardo Bolaños, DVM, Miro Moran Alvaro, DVM, Carolina Balao Da Silva, DVM, Antolin Morillo, DVM, Maria Plaza, DVM, Ines Aparicio, PhD, Jose Antonio Tapia, PhD, Cristina Ortega Ferrusola, PhD And Fernando Pena, PhD
University Of Extremadura
(Presented by: Fernando Pena, PhD)

Poster #42 **HUMAN SPERM CHROMATIN UNDERGOES REMODELING DURING INVITRO CAPACITATION AND ACROSOME REACTION: EVIDENCE FROMCYTOCHEMICAL TESTS AND CORRELATIONS BETWEEN ASSAYS**

Maria San Gabriel, PhD[1], Eve de Lamirande, PhD[2] and Armand Zini, MD[2]
[1]McGill University; [2]Urology Res Laboratory, McGill University
(Presented by: Maria San Gabriel, PhD)

Poster #43 **EXPRESSION AND BINDING CHARACTERISTICS OF RECOMBINANT MURINE BINDER OF SPERM PROTEIN HOMOLOG 2 (BSPH2A)**

Geneviève Plante[1], Claude Lazure, PhD[2] and Puttaswamy Manjunath, PhD[1]
[1]University of Montreal; [2]Institut de Recherche Clinique de Montréal
(Presented by: Geneviève Plante)

Poster #44 **SPERM DNA FRAGMENTATION AND REACTIVE OXYGEN SPECIES ESTIMATION– BETTER DIAGNOSTIC AND PROGNOSTIC MARKERS TO DISTINGUISH FERTILE AND INFERTILE MEN**

Monis Bilal Shamsi, MSc[1], Rajeev Kumar, MD[2] and Rima Dada, MD, PhD[3]
[1]A.I.I.M.S.; [2]Department of Urology, A.I.I.M.S., New Delhi, India; [3]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, A.I.I.M.S., New Delhi, India
(Presented by: Monis Bilal Shamsi, MSc)

Poster #45 **8-HYDROXY-DEOXYGUANOSINE (8-OH-DG) IN HUMAN SPERM: RELATIONSHIP WITH SEMEN QUALITY AND DNA FRAGMENTATION**

Lara Tamburrino, PhD student, Sara Marchiani, Research Assistant, Marta Cambi, PhD student, Biagio Olivito, Research Assistant, Chiara Azzari, Professor of Pediatry, Gianni Forti, Professor of Endocrinolgy, Elisabetta Baldi, Professor of Clinical Pathology and Monica Muratori, Research Assistant
University of Florence
(Presented by: Elisabetta Baldi, Professor of Clinical Pathology)

Poster #46 **IMMUNOREACTIVE SPERM ANTIGENS IN PROTEOMIC ANALYSIS AND THEIR RELEVANCE TO SPERM FUNCTION**

Karolina Nowicka-Bauer, MSc[1], Marzena Kamieniczna, ScD[1], Jan Cibulka, MD[2], Zdenka Ulcová-Gallová, MDPhD[2] and Maciej Kurpisz, MDPhD[1]
[1]Department of Reproductive Biology and Stem Cells, Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Department of Gynaecology and Obsterics, Charles University and Faculty Hospital, Pilsen, Czech Republik
(Presented by: Karolina Nowicka-Bauer, MSc)

Poster #47 **SPERM QUALITY ANALYSIS IN INFERTILE PATIENTS WITH CLINICAL AND SUBCLINICAL VARICOCELE REGARDING WITH THE SURGICAL TREATMENT**

Naim Hannaoui, Dr., Maria Jose Amengual, Dr.[1], Carlos Abad, Dr[2], Jose Luis Gonzalez, Dr.[2], Eduardo Vicente, Dr.[2], Agustín Garcia, Dr.[2] and Juan Prats, Dr.[2]
[1]UDIAT, Consorci Hospitalari Parc Taulí, Spain; [2]Servei d'Urologia, Consorci Hospitalari Parc Taulí, Spain
(Presented by: Naim Hannaoui, Dr.)

Poster #48 **ANTIOXIDANT TREATMENT (ANDROFERTI) IMPROVE DNA SPERM QUALITY IN ASTHENOTERATOZOOSPERMIC INFERTILE MALES**

Carlos Abad, Dr., Maria Jose Amengual, Dr.[1], Naim Hannaoui, Dr.[2], Angel Prera, Dr.[2], Dario Garcia, Dr.[2], Raul Martos, Dr.[2], Jesus Muñoz, Dr.[2], Agustín Garcia, Dr.[2] and Juan Prats, Dr.[2]
[1]UDIAT,Consorci Hospitalari Parc Taulí, Spain; [2]Servei d'Urologia, Consorci Hospitalari Parc Taulí, Spain
(Presented by: Carlos Abad, Dr.)

SPERMATOGENESIS / STEROIDOGENESIS / TESTIS BIOLOGY

Poster #49 **COMPARISON OF STREPTOZOTOCIN-INDUCED DIABETIC AND INSULIN RESISTANT EFFECTS ON SPERMATOGENESIS WITH PROLIFERATIVE CELL NUCLEAR ANTIGEN IMMUNOSTAINING OF ADULT RAT TESTIS**

Adesina Arikawe, BSc; MSc; MBBS; PhD (in view)[1], Biodun Oyerinde, BSc; MSc[2], Adetola Daramola, MBBS; FWAC Path[2], Ibiyemi Olatunji-Bello, BSc; MSc; PhD[3] and Leonard Obika, BSc; MSc; PhD[4]
[1]University of Lagos; [2]College of Medicine, University of Lagos; [3]Lagos State University College of Medicine; [4]College of Medical Sciences, University of Benin
(Presented by: Adesina Arikawe BSc; MSc; MBBS; PhD (in view))

Poster #50 **RHOX HOMEOBOX GENES ESSENTIAL FOR GERM CELL PROGRESSION IN VIVO**

Anilkumar Bettegowda, PhD and Miles Wilkinson, PhD
Reproductive Medicine Department, University of California, San Diego, La Jolla, CA 92093
(Presented by: Miles Wilkinson, PhD)

Poster #51 **OXIDATIVE STRESS MEDIATED UPREGULATION OF MITOCHANDRIAL DEPENDEND GERM CELL APOPTOSIS IN STREPTOZOTOCIN INDUCED DIABETIC MALE RAT**

Sekar Suresh, PhD[1] and Seppan Prakash, PhD[2]
[1]Animal Genomic and Bioinformatic Division National Institute of Animal, South Korea,; [2]Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, India
(Presented by: Sekar Suresh, PhD)

poster session I

Poster #52 **MOUSE RC/BTB2, A MEMBER OF THE RCC1 SUPERFAMILY, LOCALIZES TO THE ACROSOMAL VESICLE THROUGH ITS BTB DOMAIN IN ROUND SPERMATIDS**

Maria Eugenia Teves, PhD, Jiannan Wang, Xuening Shen, David R. Nagarkatti-Gude, Jerome F. Strauss III and Zhibing Zhang
Department of Obstetrics & Gynecology, Virginia Commonwealth University
(Presented by: Maria Eugenia Teves, PhD)

Poster #53 **TESTIS VOLUME AND THE PRESENCE OF SPERM IN THE EJACULATE OF ADOLESCENTS WITH KLINEFELTER SYNDROME**

Akanksha Mehta, MD, Alexander Bolyakov, MCSc, Peter Stahl, MD and Darius A. Paduch, MD, PhD
Department of Urology, Weill-Cornell Medical College
(Presented by: Akanksha Mehta, MD)

Poster #54 **CHARACTERIZATION OF A NOVEL TESTIS-SPECIFIC PROTEIN LOCALIZED TO THE GOLGI/ACROSOME OF POST-MEIOTIC GERM CELLS**

Edward Nguyen and Kevin Moore, MD
Oklahoma Medical Research Foundation
(Presented by: Edward Nguyen)

Poster #55 **HUMANIN PREVENTS HEAT-INDUCED GERM CELL APOPTOSIS VIA ITS COGNATE RECEPTOR IN AN EX VIVO SEMINIFEROUS TUBULE CULTURE SYSTEM**

Yue Jia, MD, PhD[1], Yanhe Lue, MD[1], Ronald S. Swerdloff, MD[1], Vince Atienza[1], Niluofar Ilani, MD[1],
Prasanth Surampudi, MD[1], Kuk Wha Lee, MD[2], Laura Cobb, PhD[2], Pinchas Cohen, MD[2] and Christina Wang, MD[1]
[1]LA BioMed; [2]UCLA
(Presented by: Yue Jia MD, PhD)

Poster #56 **ACTIVIN-HORMONE CROSSTALK IN TESTIS DEVELOPMENT AND DYSGENESIS: CLUES FROM INHIBIN ALPHA HETEROZYGOUS AND KNOCKOUT MICE**

Catherine Itman, PhD and Kate Loveland, PhD
Monash University
(Presented by: Catherine Itman, PhD)

poster session II

Monday, April 23, 2012
11:00 a.m. – 12:30 p.m.
Location: Exhibit Hall

ANDROGENS / ENDOCRINOLOGY

Poster #57 **MALE HYPOGONADISM IN A FEMALE TRANSSEXUAL**
Shant Ayanian, MD and Michael Irwig, MD
GWUH
(Presented by: Shant Ayanian, MD)

Poster #58 **MEDICALLY ALTERING THE TIMING OF RUT IN MALE PACIFIC WALRUS (ODOBENUS ROSMARUS DIVERGENS) TO ACHIEVE A PREGNANCY IN A NULLIPAROUS FEMALE**
Holley Muraco, BS[1], Leah Coombs[1], Diana Procter[1], Paul Turek, MD[2] and Michael Muraco[1]
[1]Six Flags Discovery Kingdom; [2]The Turek Clinic
(Presented by: Holley Muraco, BS)

ENVIRONMENT / TOXICOLOGY

Poster #59 **RANDOMIZED CONTROL DIETARY TRIAL OF WALNUTS ADDED TO WESTERN STYLE DIET: IMPACT ON SERUM FATTY ACID PROFILES, SPERM VITALITY, MOTILITY, AND MORPHOLOGY**
Wendie A. Robbins, PhD[1], Lin Xun, MS[2], Brittany N. Johnson, BSc[2], Susanne M. Henning, PhD[3] and Catherine L. Carpenter, PhD[3]
[1]University of California, Los Angeles; [2]UCLA School of Nursing; [3]UCLA Medicine, Clinical Nutrition
(Presented by: Wendie A. Robbins, PhD)

Poster #60 **CYCLOPHOSPHAMIDE TREATMENT AFFECTS MICRORNA EXPRESSION PROFILES IN MALE RAT GERM CELLS**
Anne Marie Downey, MSc[1], Claudia Lalancette, PhD[1] and Bernard Robaire, PhD[2]
[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada; [2]Department of Pharmacology and Therapeutics, and Obstetrics and Gynecology, McGill University, Montreal, QC, Canada
(Presented by: Anne Marie Downey, MSc)

Poster #61 **EFFECTS OF PHTHALATES ON 3β–HYDROXYSTEROID DEHYDROGENASE AND 17β–HYDROXYSTEROID DEHYDROGENASE 3 ACTIVITIES IN HUMAN AND RAT TESTES**
Ying Su, MD[1], Binghai Zhao, PhD[2], Ren-Shan Ge, MD[3] and Qing-Quan Lian, PhD[1]
[1]Wenzhou Medical College; [2]Heilongjiang Key Laboratory of Anti-fibrosis Biotherapy, Mudanjiang Medical University; [3]Population Council
(Presented by: Ying Su, MD)

Poster #62 **DIRECT INHIBITORS OF TESTICULAR 17α -HYDROXYLASE ACTIVITY**
Leping Ye, MD[1], Ren-Shan Ge, MD[2] and Qingquan Lian, MD[1]
[1]Wenzhou Medical College; [2]Population Council
(Presented by: Leping Ye MD)

EPIDIDYMIS / VAS DEFERENS / SEMINAL VESICLES

Poster #63 **LOCALIZATION OF SPAG11C IN EMBRYONIC AND POSTNATAL EPIDIDYMIS UNRAVELS ANDROGEN-DEPENDENT MECHANISMS REGULATING ITS EXPRESSION**
Camilla Ribeiro Master, Daniel Queiróz, PhD, Erick Silva, PhD, Alexandre Denadai-Souza, PhD and Maria Christina Avellar, PhD
Universidade Federal de São Paulo
(Presented by: Camilla Ribeiro Master)

Poster #64 **CIRCULATING ALDOSTERONE INDUCES THE APICAL ACCUMULATION OF THE PROTON PUMPING V-ATPASE IN CLEAR CELLS IN THE CAPUT EPIDIDYMIS**
Jeremy Roy, PhD, Teodor G. Paunescu, PhD, Dennis Brown, PhD and Sylvie Breton, PhD
Massachusetts General Hospital, Harvard Medical School, Program in Membrane Biology, Center for Systems Biology
(Presented by: Jeremy Roy, PhD)

poster session II

FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT

Poster #65 **TESTIS-SPECIFIC SERINE/THREONINE KINASE (TSSK) FUNCTION IS NECESSARY FOR SPERMIOGENESIS IN CAENORHABDITIS ELEGANS**
Matthew Marcello, PhD[1], Priyanka Vijay[2], Anam Rizvi[2], Pablo Visconti, PhD[3], Ana Maria Salicioni, PhD[3] and Andrew Singson, PhD[2]
[1]Waksman Institute, Rutgers University and UMDNJ-Robert Wood Johnson Medical School; [2]Waksman Institute, Rutgers University; [3]University of Massachusetts, Amherst
(Presented by: Matthew Marcello, PhD)

Poster #66 **ROLE(S) OF MOUSE SPERM ASSOCIATED ALPHA-L-FUCOSIDASE IN FERTILIZATION**
Kamonrat Phopin, PhD, Wutigri Nimlamool, PhD candidate and Barry Bean, PhD
Lehigh University
(Presented by: Kamonrat Phopin, PhD)

Poster #67 **ISOLATED ACROSOMAL MATRICES FROM MOUSE SPERMATOZOA CONTAIN AMYLOID-LIKE STRUCTURES AND CRES (CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC) SUBGROUP MEMBERS**
Benoit Guyonnet, PhD[1], Sandra Whelly, PhD[1], Masoud Zabet-Moghaddam, PhD[2], Susan Sanfrancisco, PhD[2] and Gail A. Cornwall, PhD[1]
[1]Texas Tech Health Sciences Center; [2]Texas Tech University
(Presented by: Benoit Guyonnet, PhD)

Poster #68 **THE CONTRIBUTION OF REGULATED NUCLEOCYTOPLASMIC TRANSPORT TO PARASPECKLE FORMATION AND ITS POTENTIAL ROLE IN TRANSLATIONAL CONTROL OF SPERMATOGENESIS**
Andrew Major, BSci, Honours[1], Cathryn Hogarth, PhD[2], Yasuyuki Kurihara, PhD[3], David Jans, PhD[1] and Kate Loveland, PhD[1]
[1]Monash University; [2]Washington State University; [3]Yokohama National Institute
(Presented by: Andrew Major, BSci, Honours)

GENETICS

Poster #69 **MEASUREMENT OF 5-METHYLCYTOSINE AND 5-HYDROXYMETHYLCYTOSINE LEVELS OVER TIME IN A DONOR POPULATION AND ABERRATIONS TO GLOBAL DNA METHYLATION QUANTITIES IN AN OLIGOZOOSPERMIC POPULATION**
Timothy Jenkins, BS, Kenneth Aston, PhD and Douglas Carrell, PhD
University of Utah
(Presented by: Timothy Jenkins, BS)

Poster #70 **ASSESSMENT OF SPERM AND LEUKOCYTE TELOMERE LENGTHS IN ASSOCIATION WITH AGE AND SEMEN PARAMETERS**
Kenneth Aston, PhD[1], Abraham Aviv, MD[2], Steven Hunt, PhD[1], Masayuki Kimura, MD, PhD[2] and Douglas Carrell, PhD[1]
[1]University of Utah School of Medicine; [2]University of Medicine and Dentistry of New Jersey
(Presented by: Kenneth Aston, PhD)

INFERTILITY / ART / MALE CONTRACEPTION

Poster #71 **ROLE OF NON-INVASIVE MARKERS IN PREDICTION OF SPERM RETRIEVAL IN NON- OBSTRUCTIVE AZOOSPERMIA**
Vasan Satya Srini, DNB – Urology[1] and Srinivas Belur Veerachari, MBBS, MS[2]
[1]Medical Director – Ankur Health Care Private Limited, Director – Manipal Andrology and Reproductive Services; [2]Fellow Andrology – RGUHS
(Presented by: Vasan Satya Srini, DNB – Urology)

Poster #72 **THE EFFECT OF THE HYPO-OSMOTIC SWELLING TEST SCORE ON MISCARRIAGE RATES**
Aniela Bollendorf and Jerome Check, MD, PhD
UMDNJ, Robert Wood Johnson Medical School at Camden
(Presented by: Aniela Bollendorf)

Poster #73 **THE CORRELATION OF THE DEGREE OF ABNORMAL SPERM MORPHOLOGY USING STRICT CRITERIA AND PREGNANCY RATES FOLLOWING INTRAUTERINE INSEMINATION (IUI)**
Jerome Check, Mary Yurashevich and Aniela Bollendorf
(Presented by: Jerome Check)

poster session II

Poster #74 **EFFECT OF AGE ON PREGNANCY RATES PER CYCLE OF INTRAUTERINE INSEMINATION FOR MALE FACTOR IN NATURAL CYCLES SUPPORTED BY LUTEAL PHASE SUPPLEMENTATION**

Jerome Check, Carrie Wilson and Aniela Bollendorf
UMDNJ, Robert Wood Johnson Medical School at Camden
(Presented by: Jerome Check)

Poster #75 **EXPERIENCE WITH PERCUTANEOUS SPERM ASPIRATION (PESA) AND INTRACYTOPLASMIC SPERM INJECTION (ICSI) FOR MAN WITH VASECTOMY: ANALYSIS OF PROGNOSTIC FACTORS FOR PREGNANCY AND LIVE BIRTH**

Marcelo Vieira, MD; Urologist[1,2], Sidney Glina, MD; Urologist[1], Paulo Perin, MD; Gynecologist[2], Elvio Tognotti, MD; Gynecologist[1], Gabriel Bastidas, Trainee[1], Christie Andraus, Embriologist[1] and Felipe Glina, Medical Student[1]
[1]Aliança dos Laboratórios de Fertilização Assistida; [2]Centro Especializado em Reprodução Humana
(Presented by: Marcelo Vieira, MD; Urologist)

Poster #76 **ROLE OF SPERM FACTORS IN IDIOPATHIC RECURRENT SPONTANEOUS ABORTION**

Kishlay Kumar, MSc, Dipika Deka, MD, Rajeev Kumar, MCh and Rima Dada, MD, PhD
AIIMS
(Presented by: Kishlay Kumar, MSc)

MALE SEXUAL FUNCTION

Poster #77 **THE NONSTEROIDAL, ANTIESTROGEN TAMOXIFEN, BLOCKS 17-BETA-ESTRADIOL INDUCED RELAXING EFFECTS THROUGH NON-GENOMIC PATHWAYS IN HUMAN AND RAT CORPUS CAVERNOSUM**

Serap Gur, PhD, Asim B. Abdel-Mageed, PhD, Suresh C. Sikka, PhD, Arthur A. Caire, MD, Ege Can Serefoglu, MD, Philip J. Kadowitz, PhD and Wayne J.G. Hellstrom, MD, FACS
Tulane University Health Sciences Center, New Orleans, LA
(Presented by: Ege Can Serefoglu, MD)

OTHER

Poster #78 **IDENTIFICATION OF A VOLTAGE ACTIVATED K+ CHANNEL IN HUMAN SPERMATOZOA BY WHOLE CELL PATCH CLAMPING**

Steven Mansell, BSc, Christopher L.R. Barratt, PhD and Stuart M. Wilson, PhD
University of Dundee
(Presented by: Steven Mansell, BSc)

Poster #79 **PROTEIN PROFILING OF SEMEN SAMPLES IN PATIENTS UNDERGOING FERTILITY EVALUATION**

Shlomit Kenigsberg, PhD, Naazish Alladin, BSC, Jonathan Zicherman, Clifford Librach, MD and Sergey Moskovtsev, MD, PhD
Create Fertility Centre
(Presented by: Shlomit Kenigsberg, PhD)

PROSTATE / TESTIS CANCER / CLINICAL UROLOGY

Poster #80 **DOES AGE AFFECT THE EFFICACY AND SAFETY OF GREENLIGHT HPS™ LASER PHOTOSELECTIVE VAPORIZATION PROSTATECTOMY (PVP)?**

Carson Wong, MD[1], Xiao Gu, MD, PhD[2], Kurt Strom, MD[3] and Massimiliano Spaliviero, MD[4]
[1]SouthWest Urology, Inc.; [2]The First Clinical Medical College at Yangzhou University; [3]University of Missouri Hospital; [4]University of Oklahoma Health Sciences Center
(Presented by: Carson Wong, MD)

Poster #81 **DOES PROSTATE CONFIGURATION AFFECT THE EFFICACY AND SAFETY OF GREENLIGHT HPS™ LASER PHOTOSELECTIVE VAPORIZATION PROSTATECTOMY (PVP)?**

Carson Wong, MD[1], Xiao Gu, MD, PhD[2], Kurt Strom, MD[3] and Massimiliano Spaliviero, MD[4]
[1]SouthWest Urology, Inc.; [2]The First Clinical Medical College at Yangzhou University; [3]University of Missouri Hospital; [4]The University of Oklahoma Health Sciences Center
(Presented by: Carson Wong, MD)

poster session II

Poster #82 **TISSUE PROTEOMICS TO DISCOVER BIOMARKERS OF TESTICULAR TUMOURS**

Mohammed Mosli, MBBS[1], Ihor Batruch, MSc[2], Christopher Smith, MSc[2], Brendan Mullen, MD, FRCPC[3], Eleftherios Diamandis, MD, PhD, FRCP(C), FRSC[4] and Keith Jarvi, MD, FRCSC(C)[5]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada.; [2]Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada.; [3]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada. Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, Canada.; [4]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada. Department of Clinical Biochemistry, University Health Network, Toronto, ON, Canada.; [5]Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Center at Mt. Sinai Hospital, Toronto, ON, Canada. Department of Surgery (Division of Urology), Mount Sinai Hospital, Toronto, ON, Canada, Department of Surgery, University of Toronto, Toronto, Canada

(Presented by: Mohammed Mosli MBBS)

SPERM FUNCTION / SEMEN ANALYSIS

Poster #83 **PRODUCTION AND CHARACTERIZATION OF RECOMBINANT HUMAN BINDER OF SPERM PROTEIN HOMOLOG 1 (BSPH1)**

Niaz Oliazadeh, PhD[1], Geneviève Plante, PhD[1], Claude Lazure, PhD[2] and Puttaswamy Manjunath, PhD[3]

[1]Department of Biochemistry, Faculty of Medicine, University of Montréal, QC, Canada; [2]Institut de recherches cliniques de Montréal (IRCM), Montréal, QC, Canada; [3]Department of Medicine, University of Montréal, QC, Canada

(Presented by: Niaz Oliazadeh, PhD)

Poster #84 **IDENTIFICATION OF SUMOYLATED PROTEINS IN HUMAN SPERM**

Margarita Vigodner, PhD[1], Edward Nieves, BSc[2], Vibha Shrivastava, PhD[3], Myrasol B. Callaway, BSc[2], Hannah Marmor, undergraduate[3] and Sholom-Ber Chernyak, undergraduate[3]

[1]Yeshiva University; [2]AECOM, Yeshiva University, Bronx, New York; [3]Yeshiva University, New York

(Presented by: Margarita Vigodner, PhD)

Poster #85 **SEMINAL REACTIVE OXYGEN SPECIES ESTIMATION: IS SINGLE READING ENOUGH? CLINICAL IMPLICATIONS**

Rima Dada, MD, PhD[1], Monis Bilal Shamsi, MSc[2], Kishlay Kumar, MSc[2], Jaypalraja Thilagavathi, MPharm[2], Jhumur Pani, MSc[2], Swetasmita Mishra, MSc[2], Kuldeep Mohanty, MSc[2] and Rajeev Kumar, MD[3]

[1]Laboratory for Molecular Reproduction and Genetics, A.I.I.M.S.; [2]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, A.I.I.M.S., New Delhi; [3]Department of Urology, A.I.I.M.S, New Delhi

(Presented by: Rima Dada MD, PhD)

Poster #86 **ASSESSMENT OF HUMAN SPERM CHROMATIN INTEGRITY AND DISTRIBUTION OF NUCLEAR SULFHYDRYL GROUPS BEFORE AND AFTER VARICOCELE REPAIR: A PROSPECTIVE TRIAL**

Naif Alhathal, MD, Maria San Gabriel, PhD and Armand Zini, MD

[1]McGill University

(Presented by: Naif Alhathal, MD)

Poster #87 **DIFFERENTIAL CYTOKINE EXPRESSION IN LEUKOSPERMIC AND NON-LEUKOSPERMIC SUBJECTS AS A NOVEL TOOL TO PREDICT TREATMENT RESPONSE**

Sruti Chandra, PhD, Sree Harsha Mandava, MD, Asim B. Abdel-Mageed, PhD, Wayne J.G. Hellstrom, MD, FACS and Suresh C. Sikka, PhD

Tulane University Health Sciences Center, New Orleans, LA

(Presented by: Sruti Chandra, PhD)

Poster #88 **SPERM KINETICS FOLLOWING VASECTOMY REVERSAL**

Amy Perkins, MS[1], Matthew Marks, BS[1], Peter Burrows, MD[2] and Sheldon Marks, MD[2]

[1]Arizona Andrology Laboratory & Cryobank; [2]International Center for Vasectomy Reversal

(Presented by: Amy Perkins, MS)

Poster #89 **CSRC IS NECESSARY FOR EPIDIDYMAL DEVELOPMENT AND IS INCORPORATED INTO SPERM DURING EPIDIDYMAL TRANSIT**

Dario Krapf, graduate student[1], Ye Chun Ruan, PhD[2], Agustina Battistone, graduate student[3], Eva Wertheimer, PhD[4], Archana Sanjay, PhD[5], Stephen Pilder, PhD[6], Patricia S. Cuasnicu, PhD[7], Sylvie Breton, PhD[2] and Pablo Visconti, PhD[8]

[1]University of Rosario; [2]Mass General Hospital, Harvard Medical School; [3]IBYME, CONICET; [4]University of Pennsylvania; [5]University of Connecticut; [6]Temple University; [7]IBYME, CONICET, Argentina; [8]Department of Veterinary and Animal Sciences, University of Massachusetts

(Presented by: Pablo Visconti, PhD)

poster session II

Poster #90 **SEMINAL QUALITY DECREASES OVER THE 9 YEARS IN SAO PAULO CITY**
Rosa Alice Monteiro, BSc, Juliana Pariz, MSc, Patrícia Pieri, PhD, Paulo Saldiva, PhD and Jorge Hallak, PhD
(Presented by: Jorge Hallak, PhD)

Poster #91 **SPERM ION CHANNELS AS TARGETS FOR MALE CONTRACEPTION**
James F. Smith, MD, MS[1], Yuriy Kirichok, PhD[2], Mitchell Rosen, MD[3], Polina V. Lishko, PhD[4]
[1]Department of Urology, University of California, San Francisco; [2]Department of Physiology University of California, San Francisco; [3]Department of Obstetrics and Gynecology, University of California, San Francisco; [4]Department of Molecular and Cell Biology, University of California, Berkeley
(Presented by: James F. Smith, MD, MS)

Poster #92 **BULL SPERM PLASMA MEMBRANE PROTEOME**
Keryn Byrne, BSc[1], Tamara Leahy, PhD[2], Michelle Colgrave, PhD[1], Ross McCulloch, BSc[1] and Michael K. Holland, PhD[1,2]
[1]CSIRO Livestock Industries; [2]University of Queensland
(Presented by: Michael K. Holland, PhD)

SPERMATOGENESIS / STEROIDOGENESIS / TESTIS BIOLOGY

Poster #93 **UPREGULATION OF MIR-630 BY HEAT SHOCK LEADS TO DECREASED SOX30 EXPRESSION IN SPERMATOGENESIS**
Yung-Ming Lin, MD, Chia-Ling Chung, MSc, Hsiu-Yen Ma, MSc and Chun-Wun Lu, PhD
Department of Urology, National Cheng Kung University, College of Medicine, Tainan, Taiwan
(Presented by: Yung-Ming Lin, MD)

Poster #94 **INITIAL CHARACTERIZATION OF TRIOSEPHOSPHATE ISOMERASE ISOZYMES IN MALE MOUSE GERM CELLS**
Takashi Ijiri, PhD, Tanya Merdiushev, BA, Meiissa Vadnais, VMD, PhD, Angel Lin, BS and George Gerton, PhD
Center for Research on Reproduction and Women's Health, Perelman School of Medicine at the University of Pennsylvania
(Presented by: George Gerton, PhD)

Poster #95 **LOCALIZATION OF PPARS AND SIRTUINS IN THE NORMAL MURINE/HUMAN TESTIS AND THEIR ALTERATIONS IN PATIENTS WITH INFERTILITY**
Yu Xiao, MSc[1], Srikanth Karnati, PhD[1], Wei Fan, MSc[1], Martin Bergmann, PhD[2] and Eveline Baumgart-Vogt, PhD[1]
[1]Institute for Anatomy and Cell Biology II, Justus Liebig University, Germany; [2]Department of Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Germany
(Presented by: Yu Xiao, MSc)

Poster #96 **CODING REGION AND 3' UTR RNA EDITING IN THE TESTIS**
Elizabeth Snyder, PhD[1], Tongjun Gu, PhD[1], Charles Connolly, PhD[2], Matthew Hibbs, PhD[1] and Robert Braun, PhD[1]
[1]The Jackson Laboratory; [2]University of Washington
(Presented by: Elizabeth Snyder, PhD)

Poster #97 **UP-REGULATION OF TESTICULAR HYPOXIA-INDUCIBLE FACTOR-1α FOLLOWING LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION**
Dharm Patel, Genevieve Fasano, Christine Dugan, BS, Marie Karpodinis, BS and Michael Palladino, PhD
Monmouth University
(Presented by: Dharm Patel)

Poster #98 **PROTEOMIC ANALYSIS OF GENE EXPRESSION IN GOLGA3<REPRO27> MALE INFERTILITY MICE**
Rahul Sigdel, BS[1], Charlotte Mobarak, PhD[2] and Carol Linder, PhD[1]
[1]NM Highlands University; [2]University of New Mexico
(Presented by: Rahul Sigdel, BS)

Poster #99 **EXPRESSION OF HEDGEHOG PATHWAY REGULATORS IN THE ADULT TESTIS REVEALS DYNAMIC CONTROL OF SIGNALLING ACTIVITY**
Kate Loveland, PhD[1], Eileen McLaughlin, PhD[2], Anette Szczepny, PhD[1] and David Jans, PhD[1]
[1]Monash University; [2]University of Newcastle
(Presented by: Kate Loveland, PhD)

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Sunday, April 22, 2012
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session I
New Discoveries on Therapeutic and Diagnostic Tools for Male Reproductive Health
Location: Turquoise I, II
Session Chairs:
Hari K. Koul, PhD
James F. Smith, MD

1

IMPROVEMENTS OF COMPONENTS OF THE METABOLIC SYNDROME UNDER TESTOSTERONE TREATMENT OVER 48 MONTHS IN THREE COHORTS, IN TOTAL, 410 MEN

Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Aksam Yassin, MD, PhD[3], Michael Zitzmann, MD, PhD[4], Louis Gooren, MD, PhD[5] and Eberhard Nieschlag, MD, PhD[4]
[1]Scientific Affairs Men's Healthcare, Bayer Pharma AG; [2]Private Urology Practice, Bremerhaven, Germany; [3]Segeberger Kliniken, Norderstedt, Germany; [4]University of Muenster, Muenster, Germany; [5]VUMC Amsterdam, Amsterdam, The Netherlands
(Presented by: Farid Saad, DVM, PhD)

Objectives: Effects of normalization of testosterone over 48 months in three cohorts of men, mostly with the metabolic syndrome, following the same treatment protocol.
Methods: Cohort 1 (MZ): 281 men (137 primary or secondary hypogonadal) (aged 40 ± 13 years), 59 with late onset hypogonadism (LOH). Cohort 2 (AH): 143 men, aged 34–78 years (mean ± SD: 62 ± 8 yrs), 119 had LOH. Cohort 3 (AY): 130 men aged 46–79 years (mean ± SD: 61 ± 9 yrs), 104 had LOH. Cut–off point was serum testosterone <12 nmol/L. Treatment with injectable testosterone undecanoate.
Results: A remarkable progressive and sustained decline of body weight and waist circumference over the full study period. Plasma cholesterol, triglycerides, and LDL–cholesterol decreased significantly over the study period. Plasma glucose declined over the first 12–18 months and then stabilized. In cohort I at baseline 240/281 men fulfilled the harmonized criteria of the metabolic syndrome, fallen to 114/281 after two years. At baseline 88/143 men in cohort 2 and 93/130 men in cohort 3 met the criteria of the metabolic syndrome by the harmonized definition. After 48 months of testosterone treatment this number had declined to 48/143 in cohort 2 and to 62/130 in cohort 3.
Conclusion: In hypogonadal men testosterone treatment over 48 months improved the metabolic syndrome with sustained declines of body weight / waist circumference along with improvements of cholesterol, LDL and triglycerides. Improvements in younger men with 'classical' primary or secondary hypogonadism (cohort 1) were of a similar magnitude as in men with LOH (cohorts 2 and 3).

2

SERUM INSL3 CONCENTRATION IS HIGHLY CORRELATED WITH INTRATESTICULAR TESTOSTERONE CONCENTRATION IN NORMAL MEN STIMULATED BY VERY LOW DOSE HUMAN CHORIONIC GONADOTROPIN DURING EXPERIMENTAL GONADOTROPIN DEFICIENCY

Mara Roth, MD[1], Kat Lin, MD[1], Katrine Bay, MSc, PhD[2], John Amory, MD, MPH[1], Bradley Anawalt, MD[1], Alvin Matsumoto, MD[3], Brett Marck, BS[3], William Bremner, MD, PhD[1] and Stephanie Page, MD, PhD[1]
[1]University of Washington; [2]Rigshospitalet; [3]Veterans Affairs Puget Sound Health Care System
(Presented by: Mara Roth, MD)

Introduction: Intratesticular testosterone (ITT) concentrations are key to stimulating spermatogenesis and may not be proportional to serum testosterone (T) in some situations. Serum biomarkers of ITT might have utility in diagnosing and monitoring treatment in infertile men. Testes–specific proteins, insulin–like factor 3 (INSL3), anti–müllerian hormone (AMH) and inhibin B, are present in serum. We assessed serum concentrations of these proteins and correlated them with ITT concentrations in normal men during experimental gonadotropin deficiency.
Methods: We induced gonadotropin deficiency in 37 normal men with the gonadotropin–releasing hormone antagonist acyline, and randomized them to receive one of four doses of human chorionic gonadotropin (hCG) – 0 IU, 15 IU, 60 IU or 125 IU subcutaneously every other day or 5 grams daily T gel for 10 days. Testicular fluid was obtained by percutaneous aspiration for measurement of androgens (T, dihydrotestosterone, androstenedione, and dehydroepiandrosterone) at baseline and after 10 days of treatment, and correlated with serum hormone concentrations. Androgen assays were completed by liquid chromatography with tandem mass–spectrometry. Serum INSL3 was quantified using time–resolved fluorescence immunoassay. Serum AMH and inhibin B were quantified using enzyme–linked immunosorbent assay
Results: At baseline, median (25th, 75th interquartile range) serum T was 4.2 (3.5, 5.0) ng/mL, serum INSL3 was 1.1 (0.8, 1.2) ng/mL, and ITT was 723 (505, 1009) ng/mL. Neither ITT nor serum T correlated with AMH, inhibin B or INSL3 at baseline. By day 10, ITT and serum INSL3 concentrations decreased by over 90% with gonadotropin suppression or gonadotropin suppression plus T gel. Serum T decreased by 97% with gonadotropin suppression and correlated highly with hCG stimulation (r 0.86, $p<0.001$). Serum INSL3 increased in a dose–dependent manner with low doses of hCG, and correlated highly with ITT ($r=0.81$, $p<0.001$). Serum AMH and inhibin B did not change with acute gonadotropin suppression, nor hCG stimulation.
Conclusion: In parallel with ITT, serum INSL3 is markedly reduced by experimentally–induced gonadotropin suppression and increased in a dose–dependent fashion with hCG stimulation in healthy men. INSL3 might be a useful biomarker of ITT concentration and Leydig cell function in response to hCG therapy, especially in the setting of T administration where INSL3 could serve as a better marker of ITT than serum T.

3

LONG-TERM SURVIVAL AND DEVELOPMENT OF XENOGRAFTED HUMAN FETAL TESTICULAR TISSUE IN IMMUNODEFICIENT MICE

Yingchun Zhu[1], Kirk Lo, MD[2], Brendan Mullen, MD[2], Keith Jarvi, MD[2] and Colin McKerlie, DVM, PhD[3]
[1]Mount Sinai Hospital; [2]Mount Sinai Hospital, Toronto; [3]Hospital for Sick Children, Toronto
(Presented by: Yingchun Zhu, MS)

Introduction and Objectives: Improved survival rates of childhood cancer calls for preservation of the male germ line in prepubertal male. Grafting of testicular tissue into immunodeficient mice has been used to achieve testicular maturation and production of sperm from immature testes in various species; however, this approach has not been successful for human testicular tissue in long term grafting. The objective of this study was to evaluate the capacity for differentiation of fetal human testicular xenografts.

Methods: Human fetal testes (at 9–12 weeks, n= 16) were grafted on male castrated NCr nude mice for 24 weeks, without (group 1) or with (group 2) human chorionic gonadotrophin (hCG) treatment of the host. Upon removal, histological and immunohistochemical analyses and competitive immunoassay for serum and xenograft tissue testosterone were used to evaluate the tissue differentiation and function.

Results: Xenografts showed 71% and 78% survival with distinct formation of seminiferous tubules in group 1 and group 2, respectively. The oncofetal protein M2A was expressed in all germ cells (gonocytes) in pre-graft tissues; while after grafting most of the germ cells had ceased to express this protein. This coincides with the emerging expression of MAGE-A4, a protein associated with differentiation into pre-spermatogonia or spermatogonia. The latter were not expressed in germ cells of pre-grafted tissues. Androgen receptor (AR) immnoexpression was absent in pre-graft tissue, but after grafting for 24 weeks AR expression was observed in peritubular myoid cells and in some Sertoli cells. Functional activation of Leydig cells was assessed in the xenografts. Treatment with hCG resulted in nearly a 20-fold increase of serum testosterone production in group 2 (mean 20.08 ± 4.3 nmol/L), compared to group 1 (mean 0.68 ± 1.2 nmol/L). Direct measurements of xenograft tissue showed similar pattern (863.6 ± 65.6 nmol/g vs 62.6 ± 13.3 nmol/g).

Conclusions: Human fetal testis tissue xenografts demonstrate normal formation of seminiferous tubules, germ cell and Sertoli cell development and testosterone production. This xenograft model is a good platform to study normal human fetal testis development. Furthermore, our model may be a powerful tool to study fetal testis susceptibility to disruption by exogenous factors, such as pharmaceutical agents and environmental toxins.

4

3D IMAGE ANALYSIS OF CHROMATIN STRUCTURE OF MOTILE AND IMMOTILE SPERM POPULATIONS

Naazish Alladin, BSc[1,2], Shlomit Kenigsberg, PhD[1], Clifford Librach, MD[1,3] and Sergey Moskovtsev, MD, PhD[1,4]
[1]CReATe Fertility Centre; [2]Department of Biomedical Sciences, Eastern Virginia Medical School, Norfolk; [3]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics & Gynaecology, Sunnybrook Health Sciences Centre; [4]Department of Obstetrics & Gynaecology, University of Toronto
(Presented by: Naazish Alladin, BSc)

Objective: Human spermatozoa contribute a haploid set of DNA to the egg for embryo development. Sperm is known to have a well-organized chromatin structure that is highly compacted, keeping nuclear chromatin more stable. Improper organization and packaging can disrupt the structured sequence of fertilization. The purpose of our study was to evaluate structural difference between motile and immotile mature human spermatozoa using 3-D digital image morphometric and image analysis.

Methods: Fresh semen sample was centrifuged using 90% density gradient medium to separate the motile and immotile populations. Cells were fixed using Carnoy's solution. Fluorescent in-situ hybridization (FISH) was used in combination with NaOH decondensation technique, to label chromosome 17 centromere and chromocenter. The volume of sperm nucleus, centromere of chromosome 17 and total chromocenter were calculated along with the intra-nuclear position of chromosome 17's centromere.

Results: The table 1 shows the results comparing motile and immotile group.

*p<0.05 immotile population has significantly higher chromosome17 centromere volume and chromocenter volume.

Furthermore, observational data was collected to show that more than 85% of the time, the centromere of chromosome 17 was found in the medial region of the sperm head, in both motile and immotile population.

Conclusion: Our data indicates that using 3-D image analysis allows us to better understand the localization of chromosomes. It can be concluded that motile and immotile population show a significant difference in the compactness of the chromatin structure. Previously, it was known that the gradient wash separates motile and immotile cells. This study shows that the gradient wash may be separating cells based on the compactness of the chromatin structure. Immotile population is prone to higher decondensation and disruption of the proper compactness.

	Motile	Immotile
Total number of cells	333	334
Volume of sperm head	70.715± 26.253	71.635± 18.106
Volume of chromosome 17 centromere	0.242± 0.167*	0.294± 0.231
Volume of chromocenter	1.539± 0.961*	2.021± 1.205
Distance between chromosome 17 and tail	2.966± 0.769	2.877± 0.877

5

VALPROIC ACID REVERSES PENILE FIBROSIS AND ERECTILE DYSFUNCTION IN CAVERNOUS NERVE INJURY RATS

Johanna Hannan, PhD, Omer Kutlu, MD, Xiaopu Liu, BS, Sena Sezen, PhD, Arthur Burnett, MD, MBA and Trinity Bivalacqua, MD, PhD
Johns Hopkins Medical Institutes
(Presented by: Johanna Hannan, PhD)

Introduction: Cavernous nerve (CN) injury causes profound penile end organ changes such as apoptosis and fibrosis. Histone-deacetylase (HDAC) has been implicated in cardiac hypertrophy and remodeling. The purpose of this study was to: 1) characterize the molecular changes in HDAC after CNI, 2) determine the effect of HDAC inhibition on markers of fibrosis, and 3) determine erectile responses to cavernous nerve stimulation (CNS).

Methods: Five groups of rats (8-10 wks) were utilized: 1) sham, 2) bilateral CNI (crush injury; 14 and 30 days), and 3) CNI treated with valproic acid [VPA (HDAC inhibitor); 14 and 30 days]. 14 and 30 days after CNI, groups underwent CNS to determine erectile function. Penile HDAC3, HDAC4, fibronectin, and transforming growth factor-β (TGF-β) protein expression (Western blot) were assessed. Trichrome staining and the fractional area of fibrosis were determined in penes from each group.

Results: There was a voltage-dependent decline ($P<0.05$) in intracavernous pressure (ICP) to CNS 14 and 30 days after CNI. Penile HDAC3, HDAC4, and fibronectin were significantly increased ($P<0.05$) 14 days after CNI. There was no change in TGF-β protein expression at either time point. Histological analysis of the penis showed increased ($P<0.05$) corporal fibrosis after CNI at both time points. VPA treatment significantly decreased ($P<0.05$) penile HDAC3, HDAC4, and fibronectin protein expression as well as corporal fibrosis. VPA-treated CNI rats had significantly ($P<0.05$) improved erectile responses to CNS at both time points.
Conclusion: HDAC-induced pathological signaling in response to CNI contributes to penile vascular dysfunction after nerve injury. Pharmacological inhibition of HDAC reverses penile fibrosis, normalizes fibronectin expression, and prevents erectile dysfunction (ED) thus the HDAC pathways may represent a suitable target in the treatment of post-radical prostatectomy ED.

6

MOLECULAR INTERACTIONS OF GALECTIN-3 AND PSA IN HUMAN SEMEN, PROSTATE, AND PROSTATE TUMORS

Alan Diekman, PhD[1], Matthew Kovak, BS, MS[2], Sabrina Goddard[2], Rebecca Gilbride, BS[2], Ashley Block, BS, MS[2] and Sarika Saraswati, PhD[2]
[1]Department of Biochemistry and Molecular Biology; [2]UAMS
(Presented by: Alan Diekman, PhD)

Prostate specific antigen (PSA) is a serine protease that is secreted by prostatic epithelial cells, liquefies the semen coagulum following ejaculation, is used as a biomarker for prostate cancer, and is implicated in the promotion of prostate cancer progression. Galectin-3 is a multivalent, carbohydrate-binding protein involved in immunomodulation, in cell adhesion, and in multiple cancers, including prostate cancer. 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 galectin-3 is a proteolytic substrate for PSA in human seminal plasma and for PSA secreted by prostate cancer cell lines. In the current study, immunoblot analysis with an anti-galectin-3 monoclonal antibody indicated that proteolytic processing of galectin-3 occurs in seminal plasma and to a lesser degree in the prostate, but not in other tissues of the male reproductive tract. Cleaved galectin-3 was also detected in purified prostasomes. Prostasomes are exosomes that are secreted by the prostatic epithelium into seminal plasma and are proposed to regulate sperm maturation and have immunosuppressive functions in the female reproductive tract. Furthermore, in vitro cleavage assays with protease inhibitors suggested that galectin-3 is cleaved by PSA in prostasomes. Immunohistochemical analysis with a small sample set of prostate tumor sections indicated that PSA co-localized with cleaved galectin-3 in prostate tumors and suggested that the extent of galectin-3 cleavage increased with tumor progression. Furthermore, the electrophoretic pattern of cleaved galectin-3 was identical to that of PSA-cleaved galectin-3 and significantly different from galectin-3 cleaved by matrix metalloprotease-2 or -9 in a small number of moderately-differentiated prostate tumors. Lectin blot analyses indicated that galectin-3 binds to the N-linked glycan on the PSA glycoprotein secreted by the normal prostatic epithelial and by prostate cancer cells; thus, PSA was identified as a glycoconjugate ligand for galectin-3. The collective results from this study suggest that PSA function in normal reproduction and in prostate cancer progression is exerted and/or regulated by molecular interactions with galectin-3.

Sunday, April 22, 2012
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session II:
Male Fertility—Spermatogenesis, Tract Biology and Sperm Function
Location: Coronado Ballroom
Session Chairs:
William Wright, PhD
Thomas Garcia, PhD

7

A SPERM STEM CELL BASED FORWARD GENETIC SCREEN FOR REPRODUCTION PHENOTYPES IN THE LABORATORY RAT

Gerardo A. Medrano, BS[1,2], Jaideep Chaudhary, BS[1,2], Heather M. Powell, BS[1,2], Karen M., Chapman BS[1,2] and F. Kent Hamra, PhD[1,2]
[1]Department of Pharmacology; [2]Cecil H. & Ida Green Center for Reproductive Biology Sciences, UT Southwestern Medical Center
(Presented by: F. Kent Hamra PhD)

Introduction: We recently established reproductive technology to help advance biomedical research through use of "Mutant Sperm Stem Cell Libraries" generated by transposon-based gene-trap insertions into cultures of spermatogonia. The study provided proof-of-principle for using custom, mobile DNA elements to "decorate" the rat genome with highly versatile gene manipulation cassettes to facilitate genome-wide gene inactivation, gene activation, exchanging DNA elements within genes, proteome tagging and the ability to repair defective genes. We chose germline stem cells from the laboratory rat to construct mutant libraries because historically, other than humans themselves, rats have been the most widely applied species to model human health.
Methods: A panel of mutant Sprague Dawley rats that harbor intronic gene-trap mutations at pre-defined loci within distinct RNA polymerase II transcribed genes was generated with the mutant sperm stem cell library. The mutant rats where then used to conduct a small-scale forward genetic screen for reproductive phenotypes. Females and males of all heterozygous mutant strains used in the screen were fertile.
Results: Currently, homozygous mutations in 15 of 19 mutant strains have been analyzed in which reproduction/developmental defects were found linked to 79% of the gene mutations. Disrupting expression of genes encoding rat Ube2k, Pan3, Btrc and Spaca6 resulted in female and/or male infertility, whereas disrupting expression of the Cdk8, Dlg1, Gsg1l, Exoc6b, Slc1a3, Slc35a3 and Txndc13 genes were each found to be embryonic lethal. Homozygous mutations predicted to disrupt expression of Pclo, Grik3, Abca13, Flstl5 and Tbc1d1 did not affect reproduction. The fertility of rats with mutations in Prkcbp1, Ube2q2 and Rgs22 is currently being analyzed.
Conclusion: This pilot study demonstrates the feasibility of using recombinant sperm stem cell libraries as a user-friendly and cost-effective strategy to advance biological research via forward or reverse genetics in the laboratory rat.
Funding: This work was supported by The Eunice Kennedy Shriver National Institute of Child Health and Human Development: R01HD036022, R01HD053889 and R01HD061575.

8

CONSTITUTIVE EXPRESSION OF ACTIVATED NOTCH1 IN SERTOLI CELLS LEADS TO A LOSS OF GDNF AND CYP26B1 EXPRESSION, AND PREMATURE GONOCYTE DIFFERENTIATION

Thomas Garcia, PhD and Marie-Claude Hofmann, PhD
Department of Comparative Biosciences, University of Illinois at Urbana-Champaign
(Presented by: Thomas Garcia, PhD)

Notch receptors and ligands have long been detected in Sertoli cells and germ cells in the developing and mature testis. However, the role of this pathway in spermatogenesis still remains entirely unknown. Our preliminary analyses demonstrated the presence of the activated form of NOTCH1 (NOTCH1 intracellular domain; NICD1) in fetal and postnatal Sertoli cells. Since Sertoli cells play an essential role in the maintenance and fate determination of germ cells, we hypothesized that perinatal alteration of Notch signaling in Sertoli cells would result in an alteration of gonocyte or spermatogonial stem cell (SSC) behavior. To this end, mutant mice constitutively expressing NICD1 in Sertoli cells were generated using Amh-cre. We found that adult male mice were infertile, had significantly reduced testis weights (~90% reduced), and had a complete absence of all germ cells, including SSCs, which occurred by postnatal day 2. Sertoli cells at all time points analyzed expressed normal levels of MKI67 and SOX9 protein in the mutant testes. However, gonocytes, which are normally mitotically inactive and MKI67 negative at E17.5, showed staining for MKI67 in mutant testes, which was not present in controls. Interestingly, we also found a significant increase in the number of gonocytes in close association with the basement membrane, indicating premature migration of these cells in comparison to controls. Furthermore, using TUNEL assay, we observed a significant increase in apoptotic cells in the mutants. Gene expression analyses demonstrated that transcripts for Sertoli cell secreted growth factors such as GDNF were down-regulated, and markers of germ cell differentiation, such as Stra8 and Rec8, were up-regulated. Immunostaining for STRA8 and SYCP3 at E15.5 and E17.5 confirmed abnormal gonocyte expression of these meiosis-specific markers in the mutant. Furthermore, CYP26B1—a pivotal male-specific, meiosis-inhibiting protein—was significantly decreased in mutant Sertoli cells at E15.5. Taken together, our results demonstrate that Notch signaling in Sertoli cells regulates Sertoli cell expression of Gdnf and CYP26B1, and suggests that Notch activation below a critical threshold is required for the maintenance of male germ cells in an undifferentiated state during fetal development. Supported by NICHD R01 HD044543 and NIEHS T32-ES007326

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HIDDEN MESSAGES: SPERM MRNA TRANSCRIPTS AS BIOMARKERS OF LOW DOSE TESTICULAR INJURY IN THE RAT

Sara Pacheco, BS, Linnea Anderson, BS, MS, Moses Sandrof, BS, Susan Hall, BS and Kim Boekelheide, MD, PhD
Brown University
(Presented by: Sara Pacheco, BS)

Background: The apical endpoints and mating studies used in animal models for reproductive toxicity testing are not practical or translatable in human assessment, which typically focuses on the analysis of semen and serum hormones. Because of these limitations, effort has been devoted to developing sperm molecular biomarkers that may better and more stably reflect sperm function.

Objective and Methods: The goal of this work was to 1) identify biomarkers of testis damage within rat sperm after sub-chronic low dose exposures to two cell-type specific testicular toxicants (0.33% 2,5-hexanedione [HD] and 5 mg/kg/d 1,2-dibromo-3-chloropropane [DBCP] for 3 months) using gene array analysis; 2) compare these biomarkers to traditional endpoints; 3) use qRT-PCR to determine the kinetics of the mRNA effect (HD; 0, 1, 2, and 3 months of exposure and 1, 2, and 3 months of post-exposure recovery); and 4) test the sensitivity of the biomarkers using a presumptive lowest-observable-adverse-effect-level exposure to another Sertoli cell toxicant (carbendazim [CBZ]).
Findings: Rats exposed to HD for 3 months had specific sperm mRNA transcript changes when compared to control using linear models for microarray data analysis ($n=134$; $q<0.05$). In addition to these molecular changes, HD-treated rats had decreased body, testes, and epididymides weights, and an increased number of stage-specific retained spermatid heads. All of these effects resolved after 3 months of post-exposure recovery. Rats exposed to DBCP for 3 months had no altered sperm transcripts. The only effects identified in the DBCP-treated rats were decreased body and epididymides weights, which also resolved after recovery. A time course was performed to determine the kinetics of the HD effect and time-dependent alterations were observed for 10 of the 29 candidate transcripts ($p<0.05$). Surprisingly, 6 of these transcripts remained significantly altered after the 3 month recovery period. The sensitivity of the transcript panel was tested after sub-chronic exposure to CBZ. There were no phenotypic changes observed after the 3 month exposure for CBZ; however, sperm mRNA content was altered in these rats, with 8 of the 29 candidate transcripts changed ($p<0.05$).
Conclusions: Sperm molecular signatures are more sensitive than traditional endpoints used to assess testicular toxicity, are predictive of low-dose testicular injury, and may be persistently altered for months after an exposure has ended.

10

REAL-TIME IN VIVO IMAGING OF ENDOCYTOSIS AND MICROVILLI EXTENSION IN EPIDIDYMAL CLEAR CELLS BY MULTIPHOTON INTRAVITAL MICROSCOPY

Ye Chun Ruan, PhD, Jeremy Roy, PhD and Sylvie Breton, PhD
Massachusetts General Hospital, Harvard Medical School, Program in Membrane Biology, Center for Systems Biology
(Presented by: Ye Chun Ruan, PhD)

Clear cells contribute to luminal acidification in the epididymis, via the proton pumping V-ATPase. We have previously shown that proton secretion in these cells is regulated via recycling of V-ATPase-containing vesicles to and from the apical membrane. Stimuli that increase cAMP activate proton secretion via V-ATPase apical accumulation, a process that is accompanied by the formation of numerous and extended microvilli. Clear cells have a high endocytic activity, secondary to V-ATPase recycling and to internalization of proteins that are shed from sperm during their maturation in the epididymis. Here, using state-of-the-art multiphoton intravital microscopy (IVM) and transgenic mice (B1-EGFP), in which EGFP is expressed in clear cells, we visualized in vivo in real-time endocytosis and microvilli extension in clear cells. To visualize endocytosis, epididymis was exposed in anesthetized mice, and rhodamin B (RhoB) was perfused into the lumen of the cauda epididymal tubule. Uptake of RhoB was observed in clear cells identified by their green fluorescence, confirming their high endocytic activity. After luminal washout, internalized RhoB progressively disappeared indicating recycling of RhoB-containing vesicles back

to the apical membrane or delivery to the degradative pathway. The effect of cAMP on microvilli extension was examined after tail vein injection of the permeant analogue, cpt-cAMP. Clear cells were dynamically visualized before and after the injection. Apical microvilli started to form and extend 15 min after the injection, with a maximal response observed after 30 min. At the end of each IVM experiment, epididymides were fixed, processed for cryosectioning, and labeled for the V-ATPase by immunofluorescence. V-ATPase-labeled microvilli were observed in clear cells treated with cAMP, confirming V-ATPase recruitment along with microvilli extension. Together, we show here for the first time two major physiological functions of epididymal clear cells in vivo, while they reside in their native environment in the intact animal.

11

CYTOPLASMIC DROPLETS FUNCTION AS AN ENERGY SOURCE ESSENTIAL FOR NORMAL SPERM EPIDIDYMAL MATURATION

Shuiqiao Yuan, MSc[1], Hui Xu, MD[2], Zhihong Zheng, MD, PhD[2] and Wei Yan, MD, PhD[1]
[1]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV, USA; [2]Department of Laboratory Animal Medicine, China Medical University, Shenyang, China
(Presented by: Wei Yan, MD, PhD)

Although the cytoplasm of spermatids is removed at the end of spermiogenesis, a tiny portion is usually retained to the sperm flagellum, which has been termed the cytoplasmic droplet (CD). In mice, CDs are mostly present on epididymal sperm and are believed to play a role in volume regulation for sperm osmolarity adaption. We, however, observed that epididymal sperm without CDs never developed motility after being collected into the HTF medium, whereas sperm with CDs were generally motile, suggesting that the CD may have a role in sperm motility competence during epididymal maturation. By analyzing mutant mice with late spermiogenic disruptions, we also found that abnormal position and morphology of CDs in epididymal sperm were associated with abnormal spermiogenesis and also with abnormal motility. Our data suggest that CDs represent a transient organelle in epididymal sperm, which are reflective of normal spermiogenesis and are predictive of sperm motility.

To further explore the function of CDs, we purified CDs (>90% purity) from epididymal sperm by discontinuous sucrose gradient centrifugation and protein contents of CDs were determined by mass spectrometry. A total of 105 proteins enriched in CDs were identified, among which 72 were enzymes involved in numerous metabolic pathways. We detected 27 glycolytic enzymes of high levels in CDs, which were involved in various steps of the glycolytic pathways, suggesting that CDs may be a site of active glycolysis and the major source of ATP production during sperm maturation in the epididymis. By investigating effects of CD removal on sperm mitochondrial activity and sperm ATP production before and after in vitro activation, we demonstrated that sperm without CDs failed to have their mitochondria activated and their capability of ATP production is minimal upon ejaculation or upon activation in the HTF medium.

Taken together, our data have revealed that CDs are enriched of glycolytic enzymes and substrates for glycolysis, and CDs are essential for mitochondrial activation and normal ATP production upon ejaculation or in vitro activation. Given that the mitochondrial activity of epididymal sperm is mostly suppressed, we propose that CDs function as a source of ATP production to facilitate all molecular and
cellular events [e.g. mitochondrial activation (mitochondrial "priming"), tyrosine phosphorylation, correct localization of glycolytic enzymes to the flagellum, etc.] during sperm epididymal maturation.

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JUNCTIONAL ADHESION MOLECULE-A (JAM-A) INTERACTS WITH CALCIUM/CALMODULIN-DEPENDENT SERINE KINASE (CASK) AND IS SERINE-PHOSPHORYLATED IN HUMAN AND MOUSE SPERM; ITS POTENTIAL ROLE IN ASTHENOZOOSPERMIA AND MALE SUBFERTILITY

Rolands Aravindan, PhD and Patricia Martin-DeLeon, PhD
University of Delaware
(Presented by: Patricia Martin-DeLeon, PhD)

We have recently shown that in mouse sperm CASK co-localizes on the flagellum with JAM-A and the major Ca2+ efflux pump, PMCA4b (Plasma membrane Ca2+/calmodulin-dependent ATPase-4b), both of which competitively bind it (via its single PDZ domain) to regulate Ca2+ and ATP homeostasis. The binding of CASK to PMCA4b inhibits Ca2+ efflux whereas its sequestration by JAM-A facilitates it. In view of the recent revelation that CASK is indeed capable of kinase activity despite lacking Mg2+-binding motif (Cell 2008, 133:328-339), the objectives of the current study were to determine if these interactions in mice also exist for human sperm and to determine if CASK-bound JAM-A is phosphorylated. We used immunofluorescence, Western blotting and co-immunoprecipitation (Co-IP) to resolve these issues. We show here for the first time that CASK is present on the human sperm flagellum and is localized on both the proximal principal piece and the midpiece where it is absent in the mouse. CASK co-immunoprecipitates with JAM-A in human sperm lysates indicating that these proteins physically interact, as in the mouse, suggesting a similar mechanism of maintenance of Ca2+ and ATP homeostasis. Co-IP studies using antibodies to CASK and phospho-Ser285-JAM-A revealed that CASK-bound JAM-A is serine-phosphorylated in both mouse and human sperm suggesting that CASK may phosphorylate not only JAM-A but also PMCA4b to which it binds. Notably, phosphorylation of JAM-A is capacitation-dependent. Western blots indicated a ~2-fold increase in Ser285-phosphorylated JAM-A in capacitated mouse sperm, compared to uncapacitated fractions; similarly, Co-IP indicated a ~6-fold increase in CASK-bound Ser285-phosphorylated JAM-A due to capacitation. Immunofluorescence of Ser285-phosphorylated JAM-A in human and mouse sperm show a wide array of capacitation-dependent localizations, even within the same sample, suggesting a dynamic spatial and temporal re-distribution of phosphorylated JAM-A, even in regions where CASK is absent. Coupled with our earlier observations that deletion of Jam-A leads to disruption of Ca2+ homeostasis and motility defects in mice and our finding of the absence of JAM-A in asthenozoospermic males attending an infertility clinic, our results suggest that CASK plays a crucial multi-regulatory role in sperm and that JAM-A may act as a signaling molecule in maintaining normal sperm motility and fertility in humans.

Funding: Supported by NIH-NCRR 2PORR015588 to P.A.M-D.

abstracts full text

Sunday, April 22, 2012
11:00 a.m. – 12:30 p.m.

Poster Session I
Location: Exhibit Hall

ANDROGENS / ENDOCRINOLOGY

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PENILE DYSMORPHOGENESIS IN RATS TREATED NEONATALLY WITH DIETHYLSTILBESTROL (DES) IS MEDIATED THROUGH STROMAL CELL REPROGRAMMING TOWARD INCREASED ADIPOGENESIS AND LOSS OF SMOOTH MUSCLE

Lilian A. Okumu[1], Liz Simon[1], Tim Braden[2] and Hari Goyal[1]
[1]Tuskegee University; [2]Auburn University
(Presented by: Lilian A. Okumu)

The objective of this study was to elucidate mechanisms by which neonatal exposure to estrogen during a critical period of development induces accumulation of fat cells and loss of smooth muscle cells in the penis. One-day-old pups were treated for six consecutive days with DES, with or without androgen receptor (AR) agonist dihydrotestosterone (DHT) or estrogen receptor (ESR) agonist ICI 182780. Testicular testosterone (T) and 17β-estradiol (E2) were assayed at 7 days. Penile tissues were examined for histopathology and for expression of Ar, Esr1, Esr2, fat cell marker (Pparg) and smooth muscle cell marker (Myh11) at day 7 and 21 using quantitative-real-time-PCR (Q-RT-PCR). The intratesticular T surge was reduced by 90% in the DES group, and the reduction was prevented by ICI co-administration, but not by DHT co-administration. Conversely, E2 level was similar to controls in all treatment groups. DES caused significant ($P \leq 0.05$) reductions in penile measurements and induced fat accumulation and loss of smooth muscle cells in the corpora cavernosa. While mRNA expression for Esr1 and Pparg was increased, that for Myh11 was decreased, and that for Ar and Esr2 was unaltered in the DES group ($P \leq 0.05$) compared to controls. Immunohistochemical localization of proteins supported Q-RT-PCR observations. Generally, DES-induced changes in histopathology and mRNA/protein expression were mitigated by co-administration with ICI or DHT. Hence, an increase in adipocyte marker and a decrease in smooth muscle marker provide evidence for the predisposition of stromal cells in the penis to differentiate into adipocytes rather than smooth muscle cells, as a result of neonatal exposure to estrogens. The mitigation of DES-induced effects by DHT and ICI suggests the involvement of both AR- and ESR1-mediated pathways. However, in the case of AR mediation, it is most likely initiated by reduced T since AR level is unaltered.

Funding: Supported by NIH/NIEHS grant 1SC1ES019355-01 (to H.G.).

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COMORBIDITIES IN MEN WITH "LATE ONSET HYPOGONADISM" TREATED IN TWO UROLOGICAL CENTRES OF COMPETENCE

Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Aksam Yassin, MD, PhD[3] and Louis Gooren, MD, PhD[4]
[1]Scientific Affairs Men's Healthcare, Bayer Pharma AG; [2]Private Urology Practice, Bremerhaven, Germany; [3]Segeberger Kliniken, Norderstedt, Germany; [4]VUMC, Amsterdam, The Netherlands
(Presented by: Farid Saad, DVM, PhD)

Introduction: It is now widely accepted that serum testosterone (T) declines with aging. At the same time it has become clear that this is not primarily determined by calendar age per se but rather by factors impairing the health of aging men, such as obesity, metabolic syndrome, diabetes mellitus and other diseases. We determined concurrent diseases in two cohorts of mainly elderly men with "late onset hypogonadism" ("LOH").

Methods: Two cohorts of men with LOH from two urological clinics were analyzed for concurrent diseases. Cohort A consisted of 230 and cohort B of 130 men.

Results: The following concurrent diseases were encountered: Cardiology: Hypertension: A: 37%, B: 58%; postmyocardial infarction: A: 13%, B: post-stroke 2%; coronary artery disease: A: 18%, B: 21%. Internal Medicine: A: type 2 diabetes: A: 27%, B: 36%. Gastroenterology: inflammatory bowel disease: A: 16%, B: 5%. Urology: chronic prostatitis: A: 30%. Dermatology: psoriasis: A: 1%, B: 2%. Nephrology: post-kidney transplant: A: 1%, B: 2%. Endocrinology: Klinefelter syndrome: A: 9%, B: 2%.Orthopedics: osteoporosis: A: 13%, B: 3%.

Conclusions: I) men with "LOH" should be diagnosed for concurrent diseases which should be adequately treated. T administration treatment may be a significant element in their treatment. 2) With progression of their age elderly men will suffer increasingly from ailments and "LOH" may be an element, so far not often diagnosed. Testosterone therapy may contribute to a better quality treatment. There is already evidence that this is the case for conditions like cardiovascular disease, diabetes mellitus, osteoporosis and Klinefelter syndrome. It can be argued for a more widespread measurement of T in elderly men not primarily seeking help for the classical conditions associated with hypogonadism, such as erectile dysfunction.

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ANDROGENIC EFFECTS OF BASIL, OCIMUM BASILICUM, ON SPERMATOGENESIS DEFICIENCY PRODUCED BY EXPOSURE TO ELECTROMAGNETIC FIELD IN RATS.

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(Presented by: Arash Khaki DVM, PhD

Introduction and Objectives: The continued development of electronic technology has led to an increased level of concern about the public health hazards of chronic exposure to electromagnetic fields (EMF).Medicinal use of basil, Ocimum basilicum, (O.B) dates back to ancient times in Iran, China, and India. This herb has been used since ancient times as a medicine and food and it is known that the antioxidant effect of (O.B) is beneficial to spermatogenesis, so it was hypothesized that this herb might also provide protection to sperm parameters from the harmful effects of EMF.

Methods: Male Wistar rats (n=40) were allocated to four groups, a control group (n=10) and three treatment groups (n=30).The first treatment group received (O.B) extract (1.5g/kg B/W), the second extract group received (O.B) extract (1.5g/kg B/W) and EMF exposure at 50 Hz for 40 consecutive days, whilst the third group received only EMF exposure for 40 consecutive days. At the conclusion of the test period rat testes tissues were removed from all group members, before sperm was collected from the epididymis and prepared for analysis.
Results: Total testosterone serum, sperm concentration, percentage of sperm viability, and sperm motility were significantly increased in the experimental group, which received (O.B) extract ($p<0.05$), compared to control and EMF treated groups.
LH, FSH hormones, morphology, and testes weights for both experimental and control groups were similar.
Conclusion: Results indicate that administration of (O.B) extract significantly increased sperm percentage, viability, motility, and total serum testosterone. This suggested that (O.B) extract may be a promising treatment for enhancing healthy sperm parameters.
Key words: Androgen, EMF, Ocimum basilicum, Sperm, Testes, Testosterone.

ENVIRONMENT / TOXICOLOGY

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HIDDEN MESSAGES: SPERM MRNA TRANSCRIPTS AS BIOMARKERS OF LOW DOSE TESTICULAR INJURY IN THE RAT

Sara Pacheco, BS, Linnea Anderson, BS, MS, Moses Sandrof, BS, Susan Hall, BS and Kim Boekelheide, MD, PhD
Brown University
(Presented by: Sara Pacheco, BS)

Background: The apical endpoints and mating studies used in animal models for reproductive toxicity testing are not practical or translatable in human assessment, which typically focuses on the analysis of semen and serum hormones. Because of these limitations, effort has been devoted to developing sperm molecular biomarkers that may better and more stably reflect sperm function.
Objective and Methods: The goal of this work was to 1) identify biomarkers of testis damage within rat sperm after sub-chronic low dose exposures to two cell-type specific testicular toxicants (0.33% 2,5-hexanedione [HD] and 5 mg/kg/d 1, 2-dibromo-3-chloropropane [DBCP] for 3 months) using gene array analysis; 2) compare these biomarkers to traditional endpoints; 3) use qRT-PCR to determine the kinetics of the mRNA effect (HD; 0, 1, 2, and 3 months of exposure and 1, 2, and 3 months of post-exposure recovery); and 4) test the sensitivity of the biomarkers using a presumptive lowest-observable-adverse-effect-level exposure to another Sertoli cell toxicant (carbendazim [CBZ]).
Findings: Rats exposed to HD for 3 months had specific sperm mRNA transcript changes when compared to control using linear models for microarray data analysis (n=134; $q<0.05$). In addition to these molecular changes, HD-treated rats had decreased body, testes, and epididymides weights, and an increased number of stage-specific retained spermatid heads. All of these effects resolved after 3 months of post-exposure recovery. Rats exposed to DBCP for 3 months had no altered sperm transcripts. The only effects identified in the DBCP-treated rats were decreased body and epididymides weights, which also resolved after recovery. A time course was performed to determine the kinetics of the HD effect and time-dependent alterations were observed for 10 of the 29 candidate transcripts ($p<0.05$). Surprisingly, 6 of these transcripts remained significantly altered after the 3 month recovery period. The sensitivity of the transcript panel was tested after sub-chronic exposure to CBZ. There were no phenotypic changes observed after the 3 month exposure for CBZ; however, sperm mRNA content was altered in these rats, with 8 of the 29 candidate transcripts changed ($p<0.05$).
Conclusions: Sperm molecular signatures are more sensitive than traditional endpoints used to assess testicular toxicity, are predictive of low-dose testicular injury, and may be persistently altered for months after an exposure has ended.

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MEHP REDUCES MAINTENANCE AND PROLIFERATION OF PRIMARY SPERMATOGONIAL STEM CELLS IN VITRO

Benjamin E. Lucas, MS and Marie-Claude Hofmann, PhD
University of Illinois – Comparative Bioscience and Institute for Genomic Biology
(Presented by: Benjamin E. Lucas, MS)

Phthalates and their metabolites are found in a number of environments, from streambeds to household dust and dairy products. Epidemiological and animal studies investigating the effects of phthalates in males provide evidence of degradation in sperm quality, numbers, and fertility, as well as an increased risk of testicular cancer. Â GDNF, or glial cell-line-derived neurotrophic factor, is a growth factor crucial for self- renewal of spermatogonial stem cells (SSCs) of the testis. GDNF is critical for continuous spermatogenesis, as GDNF-knock out testes become quickly devoid of germ cells. Alterations of GDNF signaling may alter SSC behavior and would induce both decreased sperm count and sperm quality, and may potentially lead to testicular cancer. Our previous work, using the SSC-derived cell line C18-4, showed that mono-ethylhexyl phthalate (MEHP, the main metabolite of DEHP) directly interferes with GDNF signaling components. In the present study, we investigated the effects of MEHP on primary mouse SSCs. Freshy isolated SSCs were exposed to 0, 0.5 and 5.0 μM MEHP for 24 and 48h. Quantitative PCR analysis indicated that mRNA expression levels of the transcription factor Fos and the proliferation marker Pcna were decreased in comparison to control cells, confirming the results obtained with the C18-4 cell line. Furthermore, after 24h and 48h of exposure, mRNA expression of the stem cell marker Pou5f1 (Oct4) was decreased, while the differentiation marker Tex14 was up-regulated by MEHP in comparison to control cells. These observations where confirmed by immunofluorescence. Taken together, our results suggest that MEHP reduces SSC proliferation in vitro and might shift the balance between self-renewal and differentiation toward differentiation. Therefore, in addition to damaging somatic cells, which will indirectly affect germ cells, MEHP can directly influence SSC numbers and fate. A decrease in the pool of SSCs available for maintenance of normal spermatogenesis might also explain the decrease of sperm numbers recorded in

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THE IMPACT OF COMMERCIAL AND POTENTIAL PLASTICISERS ON HUMAN PROSTATE CELL LINE FUNCTIONS

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(Presented by: Claudia Lalancette, PhD)

Animal studies showed that the current commercial plasticisers, especially phthalates, have detrimental effects on the development of the male reproductive system. Although the main focus has been on testicular development, animal and cell culture models show evidence that the prostate is also a target, but the observations are limited. As part of a multi-disciplinary project with the aim to identify safer plasticisers, we examined the impact of commercial plasticisers, as well as potential replacements, on the function of human prostate cell lines. The main objective of this study was to investigate the impact of current and potentially new plasticizers on human prostate cell lines. Human prostate cell lines LNCaP, DU145, PC3 and PNT1A were exposed to commercial plasticisers currently in use, such as Di(2-ethylhexyl) phthalate (DEHP) and 1,2-cyclohexane dicarboxylic acid diisononyl ester (Hexamoll DINCH), as well as additional compounds shown to have plasticising qualities (dibenzoate- and succinate-based). Cell viability was measured using the MTT assay. Gene expression was compared using real-time PCR. Protein expression or secretion was evaluated using Western blot. The human prostate cell lines showed different sensitivities upon exposure to the tested plasticisers. None of the cell lines were affected by succinates. Hexamoll DINCH induced a 20% decrease in cell survival for the DU145 and PC3 cell lines. Only the DU145 cell line showed a marked decrease in cell survival in the presence of dibenzoates. DEHP induced a 40% decrease in cell viability for DU145 and a slight decrease for LNCaP. Interestingly, gene expression for kalikrein 3 (PSA) in LNCaP was decreased in the presence of either phthalate or dibenzoates. For the DU145 cell line, there was an increase in the expression of the glucocorticoid receptor after incubation in the presence of dibenzoates. We conclude that succinate-based plasticisers may be safe alternatives to the current commercial plasticisers. The differences in response based on the cell lines tested suggest that when possible, multiple cell lines for the organ of interest be tested. The cell line sensitivities to the tested compounds also suggest that different pathways are involved. We are currently working on identifying those pathways that make the prostate cell lines responsive to different compounds. These studies were funded by a Team Grant from the Canadian Institutes of Health Research

EPIDIDYMIS / VAS DEFERENS / SEMINAL VESICLES

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TOXIC IMPACT OF ALUMINIUM—STUDY ON MEMBRANE BOUND ENZYMES AND SECRETORY PRODUCTS IN THE EPIDIDYMIS OF MATURE RATS

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[1]Assistant Professor; [2]Research Scholar; [3]Post Doctoral Fellow
(Presented by: Ramalingam Venugopal, PhD)

Environmental exposure to heavy metals is associated with a wide range of toxic effects. It is evident that heavy metals released in the environment affect the reproductive processes and fertility of animals. Considering the toxic nature of aluminium with less work on reproductive toxicity and the major role of plasma membrane in cellular functions and also the role of secretory products in sperm maturation, the present study was conducted to evaluate the effects of aluminium chloride on membrane bound enzymes and secretory products and its possible recovery by vitamin E treatment in the epididymis of adult rats Adult male rats were administered with aluminium chloride, 100 mg/kg body weight, orally, daily for 45 days. Second group of rats were treated with aluminium chloride along with vitamin E. Third group of rats treated with vitamin E alone and the fourth group served as withdrawal group. All the groups of rats were compared with the control group. At the end of the experimental period the animals were sacrificed and the epididymis was dissected out. The activities of adenosine triphosphatases, alkaline phosphatase, 5'nucleotidase and gamma glutamyl transferase and the secretory products like glyceryl phosphoryl cholone (GPC), sialic acid, carnitine and acetyl carnitine content were estimated by standard spectrophotometric methods. The activities of all the enzymes studied were significantly decreased. GPC, sialic acid, carnitine and acetyl carnitine content were also declined in the epididymis after aluminium treatment. The plasma membrane receives and transduces a growth stimulating signals which trigger a cascade physiological and biochemical events in the cell. Several changes take place in the spermatozoa as they pass through the epididymis. These changes are directly related to the acquisition of fertlilizing ability of spermatozoa. The results suggest that aluminium chloride has definite influence on membrane enzymes and secretory products in the epididymis and this may eventually have an impact on the fertility of the animal.

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NON CLASSICAL SECRETION BY PRINCIPAL CELLS OF THE EPIDIDYMIS

Jennifer Hughes and Trish Berger, PhD
UCDavis
(Presented by: Jennifer Hughes)

Transit of spermatozoa through the epididymis confers fertilizing ability. This involves transfer of selected proteins to the sperm plasma membrane within an aqueous environment. Protein products of non classical secretion do not have a signal sequence and do not transit the endoplasmic reticulum/Golgi complex. Apical blebbing is one of several pathways that have been identified as non classical secretion. Apical blebs from principal cells are hypothesized to be the direct source of epididymosomes and thus the source of future sperm membrane proteins. This study aims to describe the developmental pattern of apical blebbing in the porcine epididymis. Caput, corpus and caudal epididymal tissues from boars between 1 week and 40 weeks of age were fixed and evaluated for amount of blebbing using semi-quantitative techniques. Apical blebbing is evident in proximal caput tissues at two weeks of age but is not present in distal caput, corpus or cauda. Apical blebs are observed in all regions of the epididiymis in mature boars, suggesting an increase in blebbing as the cells differentiate and mature. As hormonal changes occur in the lumen of the epididymis as well as the blood of the animal, a corresponding increase in blebbing is expected. The correlation of the blebbing process with developmental markers, such as an increase in hormones or appearance of spermatozoa in the lumen, will allow future studies to mimic the hormonal and/or luminal environment within an in vitro culture, with the final goal of an in vitro apical blebbing model of epididymal cells.
Funding: Research supported in part by WK Kellogg Endowment and National Research Initiative Competitive Grant 2008-35203-19082 from the USDA National Institute of Food and Agriculture.

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EXPRESSION OF THE MAJOR SPLICE VARIANTS OF PMCA4 IN THE MURINE EPIDIDYMIS AND CAUDAL SPERM: THE ROLE OF PMCA4A IN EPIDIDYMAL MATURATION AND SPERM FUNCTION

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(Presented by: Patricia Martin-DeLeon, PhD)

Introduction: Plasma Membrane Ca2+–ATPase 4 (PMCA4) is essential for male fertility in mice where it is the major Ca2+ efflux pump in sperm. Recently, we showed that the prominent splice variant, PMCA4b, interacts with Ca2+/calmodulin–dependent serine kinase (CASK) in maintaining Ca2+ homeostasis in murine sperm. Also it has recently been shown that PMCA4, in addition to its expression in testis is expressed in the epididymis in rats and bulls where it shifts from the 4b to 4a isoform from the caput to cauda epididymis. Our goal was to determine if PMCA4a is also expressed in the murine epididymis where it is secreted and transferred to maturing sperm:
Methods: Pmca4a and 4b transcripts were analyzed in all three epididymal regions and the testis using, RT–PCR and in situ RT–PCR, while the protein was detected via immunohistochemistry using PMCA4a and pan–PMCA4 antibodies. Western analysis included proteins from caput and caudal sperm and epididymal luminal fluids. Proteins were also immunoprecipitated.
Results: Unlike bulls, 4b transcripts are uniformly expressed in the testis and all epididymal regions, while those for Pmca4a are more abundant in the testis and the cauda and lowest in the corpus. In situ RT–PCR and immunohistochemistry revealed that Pmca4a transcripts and PMCA4a are distributed in the epididymal epithelial cells and PMCA4a is most abundant in the testis and cauda. Western blotting confirmed the presence of PMCA4a in the epididymis and its abundance in the cauda and revealed an ~4–fold increase in caudal, compared to caput, sperm. Importantly, PMCA4a was detected in sperm–free epididymal luminal fluid in quantities proportional to its regional presence, suggesting that it is secreted and acquired by sperm during epididymal transit. Immunoprecipitation studies of protein extracts from caudal sperm reveal the presence of both 4a and 4b isoforms which have different properties, with respect to Ca2+ transport.
Conclusions: Murine sperm optimize the advantages of both isoforms of this 10–pass transmembrane pump in Ca2+ efflux. The recent identification of transmembrane proteins on epididymosomes from the epididymal fluids of humans and bulls, suggests that PMCA4 isoforms are present on murine epididymosomes which transfer them to sperm. Studies aimed at investigating this possibility are in progress.
Funding: Supported by NIH 1RO3 HD061637–01A1 to PAM–D.

FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT

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"POTENTIAL GENETIC BIOMARKERS IN AZOOSPERMIA BY MICROASSAY STUDIES: NEW DIMENSION IN THE EVALUATION"

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(Presented by: Vasan Satya Srini, DNB – Urology)

Introduction: To identify potential biomarkers for azoospermia by establishing the expression patterns of genes is crucial for the understanding of cell and molecular processes, under normal and disease states. Many databases are existing to provide easy access to data from such transcriptomic or proteomic studies. But, our recent study questioned the reliability of these databases in specific cases, such as mammalian testis–specific gene expression.
Objectives: To derive a novel set of candidate biomarkers for non–obstructive azoospermia (NOA). To determine a threshold for the 'reliability' of the score, which might help in identification of the potential markers (if the test results are positive).
Material and Methods: Microarray experiments.
Results: Very high number of genes present in NOA, with ≥40 score and those present in normal testis, with ≥ 6 score, were reproduced by the microarray experiment.
A new scoring system was followed. The method was efficient in determining the percentage overlap and the potential markers, wherever needed the consensus was derived from gene–lists across studies. Any block with a percentage value, greater than the expected random chance of occurrence (% overlap between the genes from the experiment with the total genes in the database), is considered as reliable block, from which the potential markers can be identified.
We developed a new method to derive a more reliable expression pattern of genes, using the existing mass–scale data – from one tissue and condition at a time. Experimental and in silico validations confirmed the higher reliability of the database. The new approach has been used to identify more dependable potential biomarkers for clinically relevant conditions.
Conclusions: A) The gene expression platform for mammalian testis provides highly reliable information in various aspects. B) Experiments and in silico analysis show that the gene expression patterns derived from the new platforms are highly reliable; the genes with higher reliability, as per the database, were repeated frequently in the experimental data set for similar conditions. C) The analysis of the experimental results also indicated a threshold level for the reliability of the score. D) New sets of potential biomarkers identified are very promising as they contain many novel genes. E) The information could be very useful for basic research as well.

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SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN RAT SPERM CAPACITATION

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(Presented by: Maria Agustina Battistone, PhD student)

Sperm capacitation relies on an increase in protein tyrosine phosphorylation mediated by PKA activation. Recent studies have suggested that Src tyrosine kinase family (SKF) is involved in capacitation-associated protein tyrosine phosphorylation either direct or indirectly. In order to elucidate between these two possibilities, we investigated whether SFK is involved in the signaling events leading to rat sperm capacitation. Western blot results showed that sperm capacitated in the presence of SFK inhibitors (SU6656 and SKI606; 50 μM), exhibited low levels of both tyrosine and PKA-substrate phosphorylation. Based on the described inhibition of ser/thr phosphatases by SFK, sperm were exposed to a ser/thr phosphatase inhibitor (okadaic acid, OA 10 nM) observing a reversion of the impaired protein phosphorylation produced by SU6656 and SKI606. However, OA was unable to induce tyrosine phosphorylation in either non capacitated sperm or sperm capacitated in the presence of the PKA inhibitor H89. Addition of both a cAMP agonist (dibutyryl cAMP, 5 mM) and a phosphodiesterase inhibitor (Pentoxifiline, 3mM) did not overcome the inhibition produced by the SFK inhibitors. In addition to their effect on PKA and protein tyrosine phosphorylation, SU6656 and SKI606 significantly inhibited capacitated sperm motility (69,4% (control) vs 42,9% (SU6656, $p<0,0001$)), vs 34,8% (SKI606, $p<0,0001$)), acrosome reaction occurrence (32,0% (control) vs 25,2% (SU6656, $p<0,0001$, vs 29,4% (SKI606, $p<0,009$)) and the sperm ability to fuse with zona pellucida-free oocytes in vitro (96,0% (control) vs 24,3% (SU6656, $p<0,0001$) vs 20,2% (SKI606, $p<0,001$)). These inhibitions were not observed when sperm were exposed to SU6656 or SKI656 in the presence of OA, indicating that the effect of the SFK inhibitors involves an up-regulation of the ser/thr phosphatase activity.
Altogether, these results support both the involvement of SFK in rat sperm capacitation and the existence of two parallel pathways leading to capacitation: one requiring cAMP/PKA activation, and the other involving the inactivation of ser/thr phosphatases.

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GENETIC INFLUENCE ON CRISP1 KNOCKOUT MICE PHENOTYPE

Mariana Weigel Muñoz, Maria Agustina Battistone, PhD student, Juan Ignacio Ernesto, PhD student, Gustavo Vasen, undergraduate, Vanina Gabriela Da Ros, PhD, Julieta Antonela Maldera, PhD, Debora Juana Cohen, PhD and Patricia S. Cuasnicu, PhD
IBYME-CONICET
(Presented by: Mariana Weigel Muñoz)

Epididymal protein CRISP1 (Cysteine-RIch Secretory Protein CRISP 1) was identified by our group and proposed to participate in the fertilization process. CRISP1-/- mice generated in our laboratory are fertile although their sperm exhibited lower levels of cAMP, and tyrosine phosphorylation and a reduced ability to interact with the zona pellucida and fuse with the oolema. Considering that different phenotypes can arise from the same mutation depending on the genetic background, in the present work we have investigated the phenotype of CRISP1-/- animals that exhibit a high degree of gene homogeneity. Mice with a mixed C57BL/6-129/SvEv background were subjected to backcrossing with C57BL/6 mice (7 cycles over two years) obtaining animals with a 99.2 % C57BL/6 background. Evaluation of fertility by natural mating revealed a phenotype different from the original hybrid colony as judged as the fact that the time of delivery was significantly greater for females mated with CRISP1-/- males than with CRISP1+/- males. Also differently from the original colony, both fresh and capacitated CRISP1-/- sperm exhibited significantly lower levels of motility which was restored by exposure of mutant sperm to a cAMP analog (db-cAMP) and a phosphodiesterase inhibitor (3-isobutyl-1-methylxanthine, IBMX). Finally, our studies revealed a significant reduction in the percentage of progesterone-induced acrosome reaction in CRISP1-/- spermatozoa compared to controls which was neither observed in the original colony. Taken together, these results revealed the relevance of CRISP1 for male fertility as well as novel roles in both sperm motility and the acrosome reaction, indicating that CRISP1-/- animals constitute a new example of the influence of the genetic background on the knockout animal phenotype.

GENETICS

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SEMEN EXAMINATION AND CFTR GENE ANALYSIS IN PATIENTS WITH CYSTIC FIBROSIS AND CBAVD SYNDROME

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(Presented by: Vyacheslav Chernykh, Dr.)

Almost all men with Cystic Fibrosis (CF) and patients with CBAVD syndrome (genital form of CF) are infertile because of obstruction of vas deferens. The aim of this study was comparative analysis of CFTR gene mutation and phenotypic variations in Cystic Fibrosis (CF) and CBAVD syndrome.
We examined 9 male patients with CF and 20 infertile men with CBAVD. Semen analysis was performed in the accordance with WHO guideline (2010). Karyological analysis of immature germ cells from ejaculate sediment was also done. Fourteen mutations and IVS8-Tn polymorphism of CFTR gene were analyzed.
Oligoasthenoteratozoospermia was detected in 5 pulmonary CF patients: moderate oligozoospermia (n=2), severe oligozoospermia with sperm count less 1 mln/ml (n=3). Azoospermia found in 3 CF patients. One 18-years-old man with pulmonary CF presents asthenoteratozoospermia. Sperm concentration 0-0.1 mln/ml was detected in all ≥20-years-old CF patients and in 1/3 18-years-old patients with pulmonary CF. Ejaculate volume was low (less 2.0 ml) in all CF patients, but volume ≤1.0 ml, and pH ≤6.5, were detected only in azoospermic and severe oligozoospermic individuals. Considerable variety in semen volume (0.3-5.5 ml) and pH (6.0-7.4) was revealed in CBAVD patients. Detailed examination of ejaculates sediment allowed to find spermatozoa and immature germ cells (at least single germ cells) in all CF patients and some of infertile men with CBAVD, including azoospermic individuals (showing incomplete obstruction), and signs of incomplete spermatogenesis arrest at prepachytene stages of prophase I meiosis in 5/9 CF patients. No leucospermia was found in both groups.
CFTR mutations and IVS8T-5T (5T) variant were found in 77.8% and 5.6% of CF alleles, 32.5% and 50% of CBAVD alleles, respectively. F508del was detected in 50% CF and 15% CBAVD chromosomes.

'Severe' genotypes were revealed only in CF patients, and 'mild' genotypes (CFTR mut/5T – 55%, and 5T/5T – 20%) were common in CBAVD. Semen parameters correlated with severity of CFTR genotypes, CF form and an age of CF individuals. Two 18–years–old pulmonary CF patients with F508del/3849+10Kb C→T genotypes present maximal sperm concentration (17.5 and 14.0 mln/ml). Both patients with pulmonary–intestinal CF present an azoospermia. Chronic bronchitis and/or pancreatic insufficiency were revealed in 20% CBAVD patients (mild form of CF?) that present severe CFTR mutation. Obtained data shown partial overlapping of CF and CBAVD.

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CLINICAL AND DIAGNOSTIC IMPLICATIONS OF LOW GRADE 46,XY/47,XXY MOSAICISM IN KLINEFELTER SYNDROME

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(Presented by: Akanksha Mehta, MD)

Introduction: The majority of Klinefelter Syndrome (KS) patients with low–grade 46,XY/47,XXY mosaicism are undiagnosed during their lifetime, and the effect of low–grade mosaicism on reproductive function, and social and cognitive development has, therefore, not been well–studied. The gold standard for KS diagnosis is karyotype analysis, which is expensive, labor– and time–intensive, and has low sensitivity for mosaicism. Fluorescence in situ hybridization (FISH) has been proposed as an alternative test for the diagnosis of KS in this setting. The aim of this study was to compare the sensitivity of FISH to that of conventional cytogenetics for the diagnosis of KS in a pair of 13–year old twin boys with differing low grades of mosaicism, and to evaluate the effect of low–grade mosaicism on testis function, fertility potential and cognitive and psychomotor development.
Methods: In addition to a complete history and physical examination, both patients underwent scrotal ultrasound, semen analyses, serum hormone evaluation, conventional karyotype analysis and FISH analysis, as well as standardized neurocognitive and neuromotor testing.
Results: Both twins were in Tanner stage III of development and appeared phenotypically different in terms of body habitus, with Twin 1 having decreased penile length and testicular volume compared to Twin 2. Conventional cytogenetic analysis revealed mosaicism in Twin 1 only. Interphase FISH analysis detected 20% and 2.2% mosaicism in Twins 1 and 2, respectively. Both boys showed a significant increase in serum FSH, characteristic of impaired testicular function in adolescents with KS. Semen analyses showed higher sperm density and motility for Twin 2 (50 vs. 18 million/mL, and 60 vs. 10%, respectively), but sperm could be cryopreserved for both patients. On standardized testing for cognitive and motor function, both boys demonstrated phenotypic features characteristic of KS. Twin 2 demonstrated significantly greater reading impairment.
Conclusion: A high index of suspicion is needed to prompt genetic testing for KS, especially in the setting of low grade 46,XY/47XXY mosaicism. Even a very low level of mosaicism (2.2%), can impact testicular function, fertility potential, and social, cognitive, and physical development. FISH can be used to confirm the diagnosis of low–grade mosaicism, allowing for early therapeutic interventions for KS patients, and improvement in their overall well–being.

INFERTILITY / ART / MALE CONTRACEPTION

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CRISP1 IN SEMINAL PLASMA: AN ALTERNATIVE FOR THE DIFFERENTIAL DIAGNOSIS OF OBSTRUCTIVE AND NON-OBSTRUCTIVE AZOOPSERMIA

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Laval University
(Presented by: Christine Legare, MSc)

Azoospermia, defined as complete absence of spermatozoa from the ejaculate, is present in less than 1% of all men and in 10 to 15% of infertile men. Azoospermia can be classified into obstructive azoospermia (OA) and non–obstructive azoospermia (NOA) according to whether there are obstructions in seminal ducts. OA patients are characterized by completed blockage in their seminal ducts resulting in the absence of spermatozoa in their ejaculates even though normal spermatogenesis. Approximately 40% azoospermic patients have OA. The other 60% do not produce spermatozoa as a consequence of spermatogenic failure; these are classified into NOA. The CRISP (Cysteine–rich secretory protein) family is a group of proteins characterized by two conserved domains and 16 conserved cysteine residues. Some of these family members have been detected in the male reproductive tract. In mammals, CRISP1 is secreted specifically by the epididymides, and binds to the post–acrosomal region of the sperm head where it plays a major role at fertilization. CRISP1 is also present in seminal plasma, but not in vasectomised men. Other glandular organs such as seminal vesicles, prostate and bulbourethral glands do not express CRISP 1 protein. In this study, we hypothesize that CRISP1 will be absent in seminal plasma of OA patients with the obstruction occurring below the epididymal level. By opposition, seminal plasma of NOA patients would contain CRISP1. Western blots analyses were performed on seminal plasma from 30 normospermic men and 52 azoospermic patients to detect CRISP 1 protein. Western blot results were compared with neutral alpha–glucosidase (NAG) enzymatic activity in seminal plasma. As expected, all normal samples were CRISP1 positive with a normal NAG activity. CRISP1 was detected in 40 azoospermic samples while twelve azoospermic patients were characterised by the absence of CRISP 1. Of the 52 azoospermic patients tested, 30 had less than normal NAG activity (<20mUml). The 12 CRISP1 negative cases were characterized by an abnormal NAG activity. Our results demonstrate that the absence of CRISP1 in the seminal plasma of azoospermic patients may contributed to a diagnosis of an obstructive azoospermia. Considering that CRISP1 is secreted specifically by the epididymides and present in the seminal plasma, it could be a good candidate for the differential diagnosis of azoospermia.
Funding: Supported by CIHR grant to RS.

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SHORTENED SPERM TELOMERE LENGTH AND ITS ASSOCIATION WITH MALE INFERTILITY

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(Presented by: Thilagavathi Jayapalraja, MPharm)

Introduction and Objectives: Telomeres are multifunctional nucleoprotein domains with repeat hexanucleotide sequences (5'TTAGGG3') in the chromosome ends. Telomeres in germ cells are balanced with each cell division and elongation by the enzyme called telomerase. Numerous studies have addressed the detailed description of telomere in various diseases but the telomere length in the sperm and its role in fertilization is not known. Therefore the current study was aimed to analysis the sperm telomere length in both infertile and control (fertile) men and to find its association with semen parameters and sperm oxidative DNA damage.
Methods: The study included 32 idiopathic infertile men and 25 fertile controls. All samples were evaluated for routine semen analysis and sperm DNA were isolated by separating the sperm from the semen by density gradient separation technique. The average mean telomere length of the sperm DNA was measured as a average ratio of telomere repeat (T) copy number to a single copy (S) gene (36B4) copy number (T/S ratio) by quantitative PCR (Q-PCR). Reactive oxygen species (ROS) level in the neat semen was estimated by chemiluminesence assay and sperm DNA integrity was analyzed by sperm chromatin structure assay (SCSA).
Results: The relative sperm mean telomere length (T/S) in infertile men was found to be significantly lower ($p<0.005$) compared to controls (0.674 ± 0.028 Vs 0.699 ± 0.030). None of the sperm parameters like sperm count, forward motility, and morphology was correlated with the sperm telomere length. Similarly no association was found between telomere length and DFI levels in idiopathic infertile men.
Conclusions: Sperm telomere length may play an important role in the fertilization and embryo development as evident from our current study. Our study for the first time confirms shortened telomere length in the sperm of infertile men may be one of the reasons behind impaired fertilization. Further study is needed to explore the role of telomere length in the male reproduction.

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ANALYSIS OF GENETIC, MOLECULAR AND LIFE STYLE FACTORS IN IDIOPATHIC INFERTILE MEN

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(Presented by: Venkatesh Sundararajan, MPharm, PhD)

Introduction and Objectives: Male infertility is one of the major reproductive health problems among the married couples and majority of them are idiopathic. Therefore the aim of the present study was to analysis genetic, molecular and various life style factors in 200 consecutive men with idiopathic infertility.
Materials and Methods: The study included cytogenetic, Y chromosome micro-deletion, sperm mitochondrial DNA mutation and sperm nuclear protein gene mutation analysis. Sperm DNA integrity and oxidative stress were also measured. A predesigned proforma were applied to record all their life style factors such as smoking, alcohol consumption, and mobile phone usage.
Results: The study revealed 13% harbored cytogenetic abnormalities, and 9.5% harboured Azoospermia factor (AZF) microdeletions. Sperm nuclear protein gene mutation screening revealed no significant difference in the frequency between infertile and controls. Fifty eight mtDNA variants were found to be common for both infertile men and control and five pathogenic mutations including ND1 (mt.3391G>A) and ND2 (mt.5186A>T) were found in 8 cases but not in controls by in silico analysis. Non-synonymous (NS) change was found to be significantly higher ($p<0.05$) in ATPase 6, ATPase 8, ND1 and CO II genes compared to ND2, CO III, ND3, ND4, and ND4L genes. Sperm DNA fragmentation index (DFI) and seminal oxidative stress in infertile men was found to be significantly higher ($p<0.0001$) compared to control men. A strong negative correlation($r= -0.260$) between testosterone level and body mass index (BMI) was observed. Smoking, alcohol and cell phone usage had moderate effect on sperm parameters. Serum prolactin level in smokers and alcoholics was found to be significantly ($p<0.01$) lower compared to non smoker and non-alcoholic men. Infertile men using cell phone for longer duration had significantly ($p<0.05$) lower sperm motility compared to non-users or moderate users.
Conclusion: Male infertility is a multi-factorial problem and our study showed more factors are involved. Cytogenetic, Y microdeletion and mtDNA variants among the genetic factors, sperm DNA fragmentation and oxidative stress and compromised life style factors play an important role in male infertility. Detailed work up of infertile men may provide a significant outcome in the treatment of male infertility.

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UTILIZATION OF FERTILITY PRESERVATION SERVICES IN NON-CANCER MEDICAL CONDITIONS

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(Presented by: Brian Le, MD)

Introduction: Introducing a formalized oncofertility program at our institution in 2006 has dramatically increased cancer patients' utilizing fertility preservation (FP) services. The program has also revealed a demand for FP in patients with non-cancer diagnoses. Some non-oncologic therapies are known to be deleterious to male reproductive potential, and the full reproductive implications of many others are unknown. To our knowledge these patients have not been documented or characterized in the literature.
Methods: For the years 2002-2010, all males ages 18-55 undergoing sperm cryopreservation or testicular tissue storage for FP purposes were identified electronically using the Enterprise Data Warehouse. A retrospective chart review was conducted to identify patient diagnoses for those undergoing FP; subsequently we looked at semen analysis reports and sperm dispositions.
Results: Of the 271 males utilizing FP services, 22 (8.1%) did so for non-cancer reasons. 4/22 (18.2%) patients were encountered before the initiation of our oncofertility program compared to 18/22 (81.8%) after. 12 patients (6 with Multiple Sclerosis) preserved their fertility prior to potentially gonadotoxic treatment for autoimmune diseases, 7 prior to non-cancer gonadal surgery, and the remaining 5 for other reasons. The average pre-treatment semen parameters for these patients were all within normal range, except for an abnormally low percent normal morphology (8.96% vs 14% Reference). Post-treatment follow-up was available on 8 patients and revealed no significant change in bulk semen parameters overall. 36.4% (8/22) of non-cancer vs. 14.8% (37/249) of cancer patients discarded their stored sperm ($p = 0.02$). To date, no non-cancer patients used their sperm samples for assisted reproduction while 8.4% (21/249) of cancer patients did.

Conclusions: Our study reveals that a significant portion of patients seeking FP may not have a cancer diagnosis, but rather have concerns over the potential deleterious reproductive effects of non-oncologic therapy. Instituting a formalized FP program allowed more of these patients opportunities to safeguard their fertility. Further research is needed to delineate where fertility preservation is appropriate in a non-cancer setting.

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RELATING ECONOMIC CONDITIONS TO VASECTOMY AND VASECTOMY REVERSAL RATES: A BI-INSTITUTIONAL PILOT STUDY

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(Presented by: Sherwin Zargaroff, MD)

Introduction: It has been theorized that utilization of permanent contraceptive methods may vary with economic conditions. Here we evaluate the relationship between both vasectomy and vasectomy reversal rates within two large referral centers in major metropolitan areas and national economic indicators over a period of two recessions.
Methods: A retrospective chart review was conducted to identify the number of vasectomies and vasectomy reversals per month at two large referral centers from January 2001 to July 2011. The incidence of these procedures were pooled and then correlated with national economic data as listed by the Bureau of Labor Statistics and other publicly available resources.
Results: There were a total of 2612 vasectomies and 359 vasectomy reversals for both institutions during the study period. The monthly vasectomy frequency correlated with the following monthly economic indicators: US Unemployment Rate ($r = +0.520$, $p<0.001$), US Leading Index ($r=+0.267$, $p=0.002$), and US Inflation Rate ($r = -0.192$, $p=0.03$). This indicates that vasectomy frequency is inversely related to the business cycle, where a higher vasectomy incidence is associated with a recessionary economy. Similarly, the annual vasectomy reversal frequency correlated with the following annual economic indicators: US Unemployment Rate ($r = -0.787$, $p=0.008$) and US Gross Savings Rate ($r = +0.810$, $p=0.006$). Thus, more vasectomy reversals are performed in an expansionary economy. Annual vasectomy and vasectomy reversal rates were inversely related ($r = -0.432$, $p=0.214$).
Conclusions: We noted a trend between the number of vasectomies and vasectomy reversals performed at our institutions and the state of the economy, with the strongest association with unemployment rate. This points to the importance of financial pressures on family planning decisions. Intuitively, the potential costs of an unplanned pregnancy in an uncertain economic atmosphere may be enough incentive for permanent contraception. Conversely, the high cost of a vasectomy reversal may be prohibitive in these economic times. Future large-scale studies are warranted to further explore this relationship.

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POST VASECTOMY REVERSAL SEMEN ANALYSIS COMPLIANCY

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(Presented by: Ryan Murphy, BS)

The average patient in the United States pays approximately $10,000, out-of-pocket, for a vasectomy reversal (VR). Despite the significant out-of-pocket expense, not all patients followed-up with a semen analysis (SA) to ensure success of the procedure. The purpose of this study was to evaluate compliancy of VR patients at one specialty center. Three hundred eighty-nine patients were retrospectively evaluated for their compliance with an advised post-operative SA. Patients were considered compliant if a pregnancy or SA result was reported. Non-compliant patients were contacted to assess their reason for failed compliance. The responses from the non-compliant patients were grouped into five categories: 1) "busy schedule;" 2) "assumed VR successful;"3) "uninterested in fertility outcome;" 4) "results left to faith;" 5) "could not locate local laboratory." The compliant group consisted of 85% (329/389) of VR patients with an average age of 39.5 years (S.D. 5.1). The non-compliant group consisted of 15% (60/389) of VR patients, with an average age of 43.9 years (S.D. 7.8). Age attributed to patient compliancy; non-compliant patients were on average 4.4 years older than compliant patients ($p<0.01$). Of the non-compliant patients reached, 17% (10/60) stated "busy schedule," as reason for non-compliancy, 10% (6/60) "assumed VR success, 10% (6/60) were "uninterested in fertility," 7% (4/60) state "results left to faith," and 2% (1/60) "could not locate local laboratory." Of the remaining non-compliant patients, 38% (23/60) received voicemails, and 17% (10/60) were unable to be reached using current contact information. The results from this study indicate that 15% of VR patients fail to comply with the advised post-operative semen analyses, which ensures success of the procedure, despite the high out-of-pocket expense. Additionally, age did contribute to compliancy rate, as non-compliant patients were on average 4.4 years older than compliant patients.

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BODY MASS INDEX DOES NOT PREDICT FOR INTRAOPERATIVE FINDINGS OR POST-OPERATIVE OUTCOMES WITH VASECTOMY REVERSAL

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(Presented by: Matthew Marks, BS)

Current studies indicate an unclear relationship between body mass index (BMI) and semen parameters. Numerous studies have cited associations between obesity and decreased sperm concentration, motility or volume yet others find no significance. The purpose of this study was to conduct a retrospective analysis of records from 405 subjects spanning two years that underwent surgery at a specialized vasectomy reversal clinic. Data included BMI, age, years since vasectomy, surgical technique (VV: vasovasostomy or VE: vasoepididymostomy or VV/VE), intraoperative vasal fluid (volume, consistency) and sperm quality assessment, and maximum total motile sperm count in post-operative semen analysis. Vasal fluid volume

was classified as copious, moderate, mild or minimal. Consistency was classified as watery, creamy or paste-like. Sperm quality was classified as best sperm seen: complete motile, complete, partial-tailed, heads or none. To assess the effect of BMI on intraoperative findings and post-operative outcomes, subjects were split into groups based on the values of BMI categories taken from the U.S. Department of Health and Human services: normal weight (BMI 18.5-24.9, n=95), overweight (BMI 25-29.9, n=207) and obese (BMI 30 or greater, n=102). The underweight category was not included due to a sample size of one. No significant differences were found between the three categories for the data assessed. All three categories had similar distributions for each parameter (see table). The average age and years since vasectomy were respectively 40 and 6 for normal BMI, 41 and 7 for overweight BMI, and 39 and 6.5 for obese BMI. The majority of subjects in each category had moderate, watery intraoperative fluid with complete or complete motile sperm and a return of sperm to the ejaculate. The range of average maximum total motile sperm per ejaculate was from 41.8 to 53.0 million with no significant difference between the BMI categories. Intraoperative vasal fluid is used as an indicator to determine optimal surgical method. BMI did not predict connection type or vasectomy reversal success.

MALE SEXUAL FUNCTION

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INCREASED SEXUAL DESIRE WITH EXOGENOUS TESTOSTERONE ADMINISTRATION IN MEN WITH OBSTRUCTIVE SLEEP APNEA: AN 18-WEEK RANDOMIZED DOUBLE-BLIND PLACEBO CONTROLLED STUDY

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(Presented by: Peter Liu, MBBS (Hons), PhD, FRACP)

Background: Sexual dysfunction, biochemical testosterone (T) deficiency, obesity and obstructive sleep apnea (OSA) often coexist. Large studies show that half of all men with OSA have erectile dysfunction, and that sexual dysfunction is common. Nevertheless, sexual dysfunction often remains undiagnosed due to patient or doctor embarrassment despite the existence of therapies which are effective in other contexts. Here we comprehensively assess the impact of T administration on sexual desire, erectile function and general and disease specific quality of life and cognitive function in obese men with OSA.

Methods: 67 middle aged (age 49±1.1, mean±SEM), obese (BMI 35.8 ± 0.57) men with moderate-severe OSA (AHI 31.8±2.4) received 3 intramuscular injections of 1000mg T undecanoate or placebo at 6 weekly intervals. SF36, FOSQ, sexual function by visual analogue scales and computerised cognitive testing were assessed at 0, 6, 12 and 18 weeks. Polysomnography (PSG) occurred at 0, 7 and 18 weeks.
Results: T administration, compared with placebo, significantly increased blood T and suppressed gonadotrophins (P<0.001). T increased sexual desire by 16% (mean difference between groups, 5.4-26.8% 95% CI, p=0.004), but did not alter erectile or orgasmic function, quality of life (FOSQ, SF-36), reaction time (PVT), spatial cognition (Tower of London) or executive memory (Stroop), irrespective of baseline T. T therapy increased vitality (p=0.004), 'feeling down' (p=0.002), and orgasmic ability (p=0.016) and reduced nervousness (p=0.032), but only in those with low baseline T. These effects did not correlate with any changes in parameters of breathing during sleep.
Conclusions: 18 weeks of T therapy improves sexual desire in obese men with OSA, and improves orgasmic function only in those with low baseline T. T therapy variably controls different facets of sexual function. However, the decision to use T therapy to improve sexual function in obese men with OSA requires consideration of both risks and benefits.

OTHER

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A NEW PROTEIN EXTRACTION PROTOCOL FOR THE PROTEOMIC STUDY OF TESTICULAR TISSUE

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(Presented by: Mohammed Mosli MBBS)

Objective: Our objective is to develop and optimize a protocol to extract proteins from freshly excised tissue for mass spectrometry (MS) analysis and utilize tissue proteomics to identify potential testicular cancer biomarkers. Up to date; there are none that are reliable (Gilligan et al, J Clin Oncol. July, 2010). The proteome of normal testicular tissue has been previously published (JY Li et al, J of Mol. & Cell Prot. 2010) with the discovery of 725 unique testis proteins. To our knowledge, proteomics has not been performed on testicular cancer.
Methods: For optimal results, tissue proteomics requires meticulously performed multi-step processing. First, we had to successfully retrieve and transfer the testicular tissue to our lab while preserving its viability and preventing protein degradation. This was achieved by using a tissue

transfer solution (phosphate buffer saline with protease inhibitor cocktail) and processing it within 20 minutes of excision. Second was depleting serum components which are high abundance proteins that typically obscure results by masking lower abundance proteins in MS detection. This was achieved by use of a haemolysis buffer (De Petris et al, Prot. Sci. 2010). Third was to optimally extract proteins for MS analysis. However, commercially available membrane detergents dramatically destabilize MS protein detection while filtering them results in loss of smaller proteins that share similar sizes as the detergent. We hence opted to use a Surfactant reagent (RapiGest™ SF) that is easily deactivated by acidification to a pH=3. The samples were then processed for fractioning using a Strong Cation Exchange column then loaded into High Pressure Liquid Chromatography coupled online to a LTQ–Orbitrap MS. Bioinformatic analysis was performed using Mascot, Xtandem and Scaffold software.

Results: We identified 2905 proteins from normal testis tissue and 1569 proteins from cancerous tissue. Few were of serum origin and none were in the 30 most abundant serum proteins indicating our success in depleting serum contaminants that could obscure lower abundance testis proteins.

Conclusions: Tissue proteomics is a complex method of tumour biomarker discovery owing to the heterogeneity of tissue. We have conducted this study on testicular cancer which is relatively homogenous compared to other tissue or tumours. We have developed a protocol that can be applied to further identify biomarkers for testicular cancer and its subtype.

PROSTATE / TESTIS CANCER / CLINICAL UROLOGY

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DOES ELEVATED BODY MASS INDEX (BMI) AFFECT THE CLINICAL OUTCOMES OF ROBOT-ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)?

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(Presented by: Carson Wong, MD)

Introduction and Objectives: With the prevalence of obesity in the United States, a significant proportion of robot–assisted laparoscopic prostatectomy (RALP) candidates have an elevated body mass index (BMI). We determine if this impacts on the clinical outcomes of the procedure using the da Vinci Si surgical system.

Materials and Methods: The hospital records of consecutive patients who underwent transperitoneal RALP by a single surgeon (CW) were reviewed. Patient demographics, clinical outcomes and adverse events were prospectively recorded in normal weight (BMI <25kg/m2), overweight (BMI ≥25 and <30 kg/m2) and obese (BMI â‰§30kg/m2) groups.

Results: 36 normal weight, 115 overweight and 67 obese patients were identified. There were no significant differences in baseline and demographic data among the three groups except for the mean BMI (23.1±1.5 vs. 27.5±1.5 vs. 32.8±2.6 kg/m2, p<0.001). The mean operative time was longer in obese patients compared to both overweight (215.4±59.7 vs. 196.5±53.6 minutes, p=0.031) and normal weight (215.4±59.7 vs. 185.0±41.8 minutes, p=0.008) patients. There were no significant differences in mean estimated blood loss (76.7±22.8 vs. 83.5±38.5 vs. 95.1±49.4 mL, p=0.315), pathologic prostate volume (44.3±12.2 vs. 45.6±13.6 vs. 44.4±14.8 mL, p=0.816), positive surgical margin rate (8.3% vs. 18.3% vs. 13.4%, p=0.313) and time to continence without pads (10.5±8.4 vs. 10.1±8.2 vs. 10.2±8.0 weeks, p=0.977). The median urethral catheter duration (5–6 days) and hospitalization (I day) were similar in all weight categories. Patients with elevated BMI had a significantly higher median Gleason score (6 vs. 6 vs. 7, p=0.046) and incidence of pathologic pT3 disease (5.6% vs. 21.7% vs. 11.9%, p=0.038). Increased BMI did not affect voiding parameters (AUASS and QoL) pre– and postoperatively (p>0.05). The incidences of adverse events including prolonged urine leak, blood transfusion, pelvic hematoma, deep vein thrombosis and bladder neck contracture were low and there were no significant differences between the three groups (p>0.05).

Conclusion: Elevated BMI appears to increase RALP operative time only, as it has little impact on other intraoperative parameters, clinical outcomes or patient morbidity. RALP is a safe and effective treatment option for clinically localized prostate cancer patients with elevated BMI.

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BLADDER NECK PRESERVATION WITH A RUNNING VESICOURETHRAL ANASTOMOSIS AND URINARY CONTINENCE FOLLOWING ROBOT-ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)

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(Presented by: Carson Wong, MD)

Introduction and Objectives: Urinary incontinence is one of the major prostate cancer treatment–related morbidities. We review our initial robot–assisted laparoscopic prostatectomy (RALP) experience with the daVinci Si surgical system, focusing on post surgery continence rates.

Materials and Methods: The hospital records of consecutive patients who underwent transperitoneal RALP by a single surgeon (CW) were reviewed. A bladder neck sparing dissection was preferentially performed during transperitoneal RALP. The urethrovesical anastomosis was completed using a running double–armed 3–0 Monocryl suture. On postoperative day 5 or 6 (clinic logistics), the urethral catheter was removed following a normal cystogram.

Results: 233 patients had a mean age of 62.7±6.7 years and serum PSA of 6.2±4.6 ng/mL. Mean operative time was 197.8±53.3 minutes and estimated blood loss was 85.4±40.0 mL. 3 (1.3%) patients required bladder neck reconstruction, while 198 (85.0%) had bilateral, 20 (8.6%) had unilateral and 15 (6.4%) did not undergo nerve sparing prostatectomy. 199 (85.4%) patients had negative surgical margins. Median hospitalization and urethral catheter duration were 1.0 and 5.0 days, respectively. At 6 weeks, a mean 1.4±1.5 pad usage per day was reported and AUASS (9.7 vs. 5.7, p=0.001) and QoL (1.9 vs. 1.4, p=0.001) were significantly improved from baseline (p<0.05). 68.8% of the patients achieved urinary continence without pads at the 3 month follow–up interval and 98.3% of patients were continence at 12 months. Age, BMI, baseline AUASS/ QoL and prior TUR surgery did not have a significant impact on the time to urinary continence (p>0.05), while patients with smaller prostates (<50 mL) were noted to achieve urinary continence earlier (9.2 vs. 12.9 weeks, p=0.007). As well, patients <70 years of age and those with smaller prostates had significantly higher continence rates during the first 3 months after surgery (p<0.05). Patients having prior TURP had markedly decreased continence rates at the 6 month follow up interval (62.5% vs. 93.5%, p=0.018), while the BMI and nerve sparing technique did not appear to impact the recovery of continence.

Conclusion: Our results suggest that a bladder neck sparing dissection in combination with a running 3–0 monocryl vesicourethral anastomosis allows for early return of urinary continence following RALP.

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BASELINE SEXUAL HEALTH INVENTORY FOR MEN (SHIM) PREDICTIVE OF ERECTILE FUNCTION FOLLOWING ROBOT-ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)

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(Presented by: Carson Wong, MD)

Introduction and Objectives: Preoperative erectile function and age are powerful predictors of erectile function following surgical treatment of clinically localized prostate cancer. We determine if baseline SHIM scores are predictive of erectile function following nerve-sparing RALP.
Materials and Methods: The hospital records of consecutive patients who underwent transperitoneal RALP by a single surgeon (CW) were reviewed. Using an anterior approach, a bladder neck sparing procedure was preferentially performed. Bilateral or unilateral nerve sparing prostatectomy was performed when appropriate. Penile rehabilitation [phosphodiesterase-5 (PDE-5) inhibitor and vacuum erection device (VED)] was offered to all patients. SHIM scores were obtained at baseline, 6 weeks and 3, 6, 9, 12, 15, 18, 21 and 24 months post surgery.
Results: 233 consecutive patients were identified, of whom 218 (93.6%) underwent nerve sparing prostatectomy [198 (85%) bilateral; 20 (8.6%) unilateral] and 15(6.4%) did not. Of those undergoing nerve sparing prostatectomy, 162/218 (74.3%) had post-surgery penile rehabilitation [phosphodiesterase-5 inhibitor ± vacuum erection device (VED)]. 11 of 162 patients were excluded due to lack of a preoperative SHIM record. The mean baseline SHIM of patients with nerve sparing prostatectomy and penile rehabilitation was 17.9 ± 7.0. 81/151 (53.6%) patients had baseline SHIM < 21 (mean 12.8±5.7), of whom 23/81 (28.4%) reported sexual potency 12 months post surgery; 70/151 (46.4%) patients had baseline SHIM ≥ 21 (mean 23.9±1.3), of whom 32/70 (45.7%) reported sexual potency 12 months post surgery. Those with a preoperative SHIM ≥ 21 achieved a significantly greater sexual potency rate versus those with a preoperative SHIM < 21 (45.7% vs. 28.4%, p=0.0041) during the follow up interval. In comparing these patient groups, SHIM postoperatively were similar until the 12 month follow-up interval, where the SHIM diverged significantly.
Conclusion: Preoperative SHIM is a useful tool in predicting postoperative erectile function in patients undergoing unilateral or bilateral nerve sparing RALP with penile rehabilitation therapy post surgery.

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DOES SIZE REALLY MATTER? THE IMPACT OF PROSTATE VOLUME ON THE EFFICACY AND SAFETY OF GREENLIGHT HPS™ LASER PHOTOSELECTIVE VAPORIZATION PROSTATECTOMY

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(Presented by: Carson Wong, MD)

Purpose: To evaluate the efficacy and safety of GreenLight HPSä laser photoselective vaporization prostatectomy (PVP) for the treatment of benign prostatic hyperplasia (BPH) in patients with different prostate volumes.
Material and Methods: Between July 2006 and February 2011, 207 consecutive patients were identified from a prospectively maintained urologic database. Based on preoperative transrectal ultrasound (TRUS) measured prostate volume, patients were stratified into two groups: â‰§80 mL (group I, n=57) and <80 mL (group II, n=150). Transurethral PVP was performed using a 120W GreenLight HPS™ side-firing laser system. American Urological Association Symptom Score (AUASS), Quality of Life (QoL) score, maximum flow rate (Qmax) and post void residual (PVR) volume were measured preoperatively and at 1 and 4 weeks and 3, 6, 12, 18, 24 and 36 months postoperatively.
Results: Among the preoperative parameters evaluated, there were significant differences (p<0.05) in the incidence of preoperative urinary retention (I: 24.6%; II: 7.3%), serum PSA (I: 4.5±2.7; II: 1.8±1.9 ng/mL), QoL (I: 4.2±1.1; II: 4.7±0.9) and mean prostate volume (I: 118.1±37.9; II: 48.5±15.5 mL), while AUASS, Qmax and PVR were similar (p>0.05) between groups. Significant differences (p<0.05) in laser utilization (I: 22.8±13.3; II: 10.4±6.4 minutes) and energy usage (I: 152.7±90.6; II: 70.9±44.8 kJ) were also noted. Clinical outcomes (AUASS, QoL, Qmax and PVR) showed immediate and stable improvement from baseline (p<0.05) within each group, but no significant differences between the two groups were observed during the follow-up period (p>0.05). The incidence of adverse events were low and similar in both cohorts.
Conclusions: These results suggest that prostate volume has little effect on the efficacy and safety of GreenLight HPS™ laser PVP, and that this technique remains a viable surgical option for BPH, irrespective of preoperative prostate volume.

SPERM FUNCTION / SEMEN ANALYSIS

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THERAPEUTIC EFFECT OF MUCUNA PRURIENS (LINN.) ON DIABETES INDUCED MITOCHONDRIAL DYSFUNCTION AND DNA DAMAGE IN RAT SPERM

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(Presented by: Seppan Prakash, PhD)

Introduction: Our previous studies were shown that the Mucuna pruriens (M. pruriens) (a leguminous plant) is a good sexual enhancer, increasing sperm count and motility in normal rat and reverse or protect aged rat sperm from oxidative damage.
Objectives: To study the effect of ethanolic seed extract of M. pruriens on mitochondrial dysfunction and DNA damage of epididymal spermatozoa in hyperglycemic rat.
Methods: Male Wistar albino rats were divided as group I (Sham) control, group II (STZ) diabetes induced [streptozotocin 60 mg/kg of body weight (b.w.) in 0.1 M citrate buffer], group III (STZ+MP) diabetic rats administered with 200 mg/kg b.w. of ethanolic extract of M. pruriens seed and group IV (Sham+MP) administered with 200 mg/kg b.w. of extract alone. M. pruriens was given (gavage) once daily for a period of 60 days. At the end of 60 days, the animals were sacrificed by cervical dislocation; the epididymal sperms were collected and subjected to various analyses.
Observations: The STZ rat sperm showed significant reduction in the sperm count, motility, viability and significant increase in the abnormal sperm compared to sham. Also the STZ group showed significant increase in free radical production, Lipid peroxidation and DNA damage, compared to Sham.

The enzymic and non-enzymic antioxidant levels, mitochondrial membrane potential and mitochondrial functions were found to be significantly reduced in STZ when compared to sham. Diabetic rats supplemented with M. pruriens extract has presented good sperm parameters and protected the mitochondrial structure and function when compared to STZ rats.
Conclusion: Sperm in STZ group appears to be having good morphology but with their defective mitochondria and damaged DNA, they could be infertile. From the overall analyses of these data's it is concluded that M. pruriens played crucial role in protecting structural and functional integrity of sperm mitochondria and DNA integrity by reducing diabetic induced OS. This study is encouraging to perform similar studies in men with diabetes induced infertility and M. pruriens may produce huge beneficial impact on improving fertilization and pregnancy rates in diabetic.

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LC3B PROCESSING AND AN APOPTOSIS LIKE MECHANISM MAY HAVE A ROLE IN THE SURVIVAL OR DEATH OF STALLION SPERMATOZOA DURING CONSERVATION IN REFRIGERATION

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(Presented by: Fernando Pena, PhD)

Apoptosis has been recognized as cause of sperm death during cryopreservation and a cause of infertility in humans, however there is no data on its role in sperm death during conservation in refrigeration; autophagy has not been described to date in mature sperm. We investigated the role of apoptosis and autophagy during cooled storage of stallion spermatozoa. Samples from seven stallions were split, half of the ejaculate was processed by single layer centrifugation, while the other half was extended unprocessed, and stored at 5ºC for five days. During the time of storage, sperm motility (CASA, daily) and membrane integrity (flow cytometry, daily) were evaluated. Apoptosis was evaluated on days 1, 3 and 5 (active caspase 3, increase in membrane permeability, phosphatidylserine translocation and mitochondrial membrane potential) using flow cytometry. Furthermore LC3B processing was investigated by western blotting at the beginning and at the end of the period of storage. The decrease in sperm quality over the period of storage was to a large extent due to apoptosis; single layer centrifugation selected non apoptotic spermatozoa, but there were no differences in sperm motility between selected and unselected sperm. A high percentage of spermatozoa showed active caspase 3 upon ejaculation, and during the period of storage there was an increase of apoptotic spermatozoa but no changes in the percentage of live sperm, revealed by the SYBR-14/PI assay, were observed. LC3B was differentially processed in sperm after single layer centrifugation compared with native sperm. In processed sperm more LC3B-II was present than in non processed samples, furthermore in non processed sperm there was an increase in LC3B-II after five days of cooled storage. These results indicate that apoptosis plays a major role in the sperm death during storage in refrigeration and that autophagy plays a role in the survival of spermatozoa representing a new pro-survival mechanism in spermatozoa not previously described.

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HUMAN SPERM CHROMATIN UNDERGOES REMODELING DURING INVITRO CAPACITATION AND ACROSOME REACTION: EVIDENCE FROMCYTOCHEMICAL TESTS AND CORRELATIONS BETWEEN ASSAYS

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Background: Capacitation (CAP) and acrosome reaction (AR) are sequential processes of sperm activation. Beside the known ionic, membrane and transduction events and final release of proteolytic enzymes to help sperm movement towards the egg, changes to chromatin are also expected. These should involve chromatin remodeling rather than damage. Our aims were to ascertain that CAP/AR does not induce DNA damage and to evaluate changes in human sperm head using cytochemical stains.
Methods: Percoll-washed spermatozoa from donors were incubated in BWW ± fetal cord serum ultrafiltrate (10%; 3.5h; CAP) and, then, with lysophosphatidylcholine (2.5 µM; 30 min; AR). Ethanol-fixed spermatozoa were used for: AR determination, aniline blue (AB, for histones), chromomycin A3 (CMA3, for protamines), toluidine blue (TB, for chromatin compaction), iodoacetamide-fluorescein (IAF, for sulfhydryl groups), induced decondensation, sperm chromatin structure assay (%DNA fragmentation index, % DFI) and immunoblotting (for histones).
Results: CAP/AR was associated with similar increases in AB (~70%) and TB (~40%) staining but had no influence on CMA3 staining. The increase (~40%) in IAF staining observed during CAP was absent after AR. CAP/AR did not damage DNA (%DFI remained low) nor affect histone content. CAP, and even more AR, primed sperm heads to decondense (~80% and ~140% increases, respectively) when challenged with 1% SDS + 0.1 mM DTT. Interestingly, induced decondensation correlated with all other tests CAP, AB, TB and IAF; also CAP was related to AB and TB stains.
Conclusions: These data strongly support human sperm chromatin remodeling during CAP/AR and that modifications are probably interlinked and help prepare chromatin for post-fertilization events.

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EXPRESSION AND BINDING CHARACTERISTICS OF RECOMBINANT MURINE BINDER OF SPERM PROTEIN HOMOLOG 2 (BSPH2A)

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(Presented by: Geneviève Plante)

Capacitation is a maturation step that is deemed to be essential for sperm to fertilize oocytes. A family of proteins, the Binder of Sperm (BSP), are known to bind choline phospholipids on sperm membranes and thus promote capacitation in many species. These proteins, secreted by the seminal vesicles, share similar characteristics such as binding to gelatine, heparin and glycosaminoglycan. BSP-homologous genes have also been identified in humans, primates and rodents. Interestingly, in these species BSP genes are expressed in epididymis rather than in seminal vesicles. BSP genes in human (BSPH1) and mice (Bsph1) have been cloned and characterized. Recent studies have demonstrated that a recombinant Bsph1 could bind to heparin, gelatine, phosphatidylcholine-liposomes and promote sperm capacitation. The objective of the current study was to determine if Bsph2a, the other murine BSP protein, plays a similar role in sperm function. To do

so, we produced recombinant Bsph2a (rec-Bsph2a) using Rosetta-gami B(DE3)pLysS cells to help with the folding of the proteins in combination with a pET32a vector which adds a thioredoxin tag to the expressed proteins which makes them more soluble. We used an immobilized metal ion affinity chromatography technique and obtained a good yield of pure proteins. The tag-free Bsph2a was obtained by treatment with enterokinase and the identity of Bsph2a was confirmed by N-terminal sequencing and mass spectrometry. The rec-Bsph2a (with or without tag) cross-reacted with anti-BsphI antibodies. The preliminary data suggests that the rec-Bsph2a can bind to heparin similar to BsphI. The availability of the tag-free protein should aid in establishing its function(s).

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SPERM DNA FRAGMENTATION AND REACTIVE OXYGEN SPECIES ESTIMATION– BETTER DIAGNOSTIC AND PROGNOSTIC MARKERS TO DISTINGUISH FERTILE AND INFERTILE MEN

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(Presented by: Monis Bilal Shamsi, MSc)

Introduction: Conventional semen analysis is now recognized to be of limited value in determination of the couples' fertility status. Oxidative stress (OS) associated sperm DNA damage are implicated in reduced fertility, impaired embryo cleavage and higher miscarriage rates.

Though numerous studies have reported sperm DNA damage and OS related factors as reactive oxygen species (ROS) and antioxidants in infertility, the inclusion of sperm DNA damage as a diagnostic test has not been addressed satisfactorily by them. This study was planned to elucidate the cut-off or threshold levels of ROS and sperm DNA damage, which could distinguish the fertile and the infertile men.

Material and Method: Semen from 286 idiopathic infertile men and 187 fertile controls was collected after 72-96 hours of sexual abstinence and analyzed according to WHO 2010 guidelines. ROS in neat semen was quantified by luminol induced chemiluminescence. TAC was assessed by commercially available kit. Sperm DNA damage and thereby DNA fragmentation index (DFI) was assessed by sperm chromatin structure assay (SCSA). Receiver operating characteristic (ROC) curve analysis was applied to find the cut-off value of ROS and DFI to distingusih infertile men from fertile men. Spearman correlation coefficient was used to test associations. P-value <0.05 was considered significant. Statistical analysis was done by MedCalc software.

Results: Mean ROS levels in infertile men was significantly higher as compared to controls ($p<0.05$). On basis of ROC analysis it was observed that 1593 RLU/min/20 million sperm is the cut off level for ROS in infertile men and controls. The average mean DFI in infertile men was found to be 39.78, as compared to 17.57 in control men. ROC analysis for DFI revealed a cut off of 28.35 % , therefore men with sperm DNA damage greater than 28.35 % are most likely to be infertile. The mean TAC levels in infertile men were significantly lower as compared to controls ($p<0.05$). On applying spearman correlation analysis it observed that sperm DNA damage was positively correlated ($r=0.712$; $p= 0.019$) with ROS and negatively correlated ($r= -0.812$; $p=0.021$) with TAC levels.

Discussion: ROS and sperm DNA damage assessment are better diagnostic and prognostic markers for reproductive capacity of sperm. Therefore including them in infertility workup may help to understand the underlying causes of infertility and also to guide the clinician in deciding the most appropriate thereupatic option.

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8-HYDROXY-DEOXYGUANOSINE (8-OH-DG) IN HUMAN SPERM: RELATIONSHIP WITII SEMEN QUALITY AND DNA FRAGMENTATION

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Oxidative stress is involved in male infertility, impacting sperm motility and viability. To investigate the impact of oxidative stress on sperm biology and male infertility, we determined the amount of sperm exhibiting 8-OH-dG in the ejaculate. We compared two methods to detect 8-OH-dG damage: an immunofluorescence technique (by using a specific antibody to 8-OH-dG) and the commercial OxyDNA kit which employs a FITC conjugated binding protein. The percentage of sperm bearing the oxidative damage in DNA was evaluated by flow cytometry. We tested the specificity of the two techniques by pre-adsorption with standard 8-OH-dG. Whereas pre-adsorption of the antibody blunts 8-OHdG fluorescent signal (basal=8.9±6.9% vs pre-adsorption=1.6±1.1%, n=3), that detected by Oxy DNA test was not affected (basal: 77.9±16.5 % vs pre-adsorption=86.4±7.5 %, n=3), indicating lack of specificity of this method. Hence the immunofluorescence technique was employed in the next experiments. The amount of 8-OH-dG was determined in the two sperm populations PIbrighter and PIdimmer identified by our group by their different nuclear staining with propidium iodide (Muratori et al, 2008). We found that only PIbr sperm exhibited 8-OH-dG (10.6±7,6%, n=98), whereas virtually no spermatozoa in PIdim population show it. We found that the percentage of sperm with 8-OH-dG negatively correlated to sperm count ($r=-0.2$, $p<0.05$), concentration ($r=-0.2$, $p<0.02$), motility ($r=-0.2$, $p<0.04$) and morphology ($r=-0.3$, $p<0.02$). To assess the relationship between oxidative DNA damage and DNA fragmentation, in 26 out of the 98 tested semen samples, we determined TUNEL positivity. No relationship between the percentage of sperm with 8-OH-G and that of sperm with DNA fragmentation was found. Moreover, by simultaneously evaluating 8-OH-G and TUNEL by fluorescent microscopy in the same semen samples, we did not detect co-localization of the two signals.

In conclusion this study demonstrates that oxidative DNA damage negatively correlates with semen parameters, suggesting that oxidative stress impacts semen quality. 8-OH-dG damage does not co-localize and is not related with DNA breakage in spermatozoa, suggesting that the two types of damages should be concomitantly evaluated to get a picture of their impact on semen quality. Finally our study demonstrated lack of specificity of the OxyDNA commercially available kit, which is currently employed by many research groups to determine theoccurrence of 8-OH-dG.

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IMMUNOREACTIVE SPERM ANTIGENS IN PROTEOMIC ANALYSIS AND THEIR RELEVANCE TO SPERM FUNCTION

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(Presented by: Karolina Nowicka-Bauer, MSc)

Introduction: The existence of antisperm antibodies (ASA) is a well known clinical fact which is estimated to affect 9–13% of infertile couples. These antibodies can be found directly bound to sperm as well as in body fluids e.g. in seminal plasma and blood. During routine analysis for detection of ASA it can be easily observed that they bind to different topographical regions of sperm recognizing various sperm entities. Different patterns of antigens recognition are also clearly visible in immunoblot pictures from numerous reports. The aim of our study was to select groups of ASA–positive sera samples of infertile couples dividing the antigens into categories relevant to sperm function.
Methods: For purpose of the study we used swim–up spermatozoa from normozoospermic ASA–negative donors. The isolation of sperm proteins was carried out by the use of commercially available protein extraction kit based on protein phase separation. Human sera samples were obtained from infertile individuals with positive results of the indirect immunobead–binding test (idIBT) or the mixed antiglobulin reaction test (MAR). ASA–negative individuals constituted controls. Two–dimensional electrophoresis (2–DE) was applied as a method of sperm proteins resolution and Western blot connected with ECL–visualization as a method of identification of immunoreactive antigens. The spots recognized by ASA were excised from 2–D gels and subjected to mass spectrometry analysis.
Results: ASA–positive sera samples recognized range of sperm specific antigens. Among identified antigens were found heat shock proteins (HSP60), proteins connected with sperm motility (fumarate hydratase), sperm metabolism (asparaginase like protein) as well as proteins directly connected with fertilization (P36–antigen).
Conclusions: Proteomic analysis may indicate sperm antigens specifically recognized by ASA. Detected antigen–antibody reactions prove the importance of some antigens for fertilization process as some of them were connected with sperm–oocyte interaction and sperm motility

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SPERM QUALITY ANALYSIS IN INFERTILE PATIENTS WITH CLINICAL AND SUBCLINICAL VARICOCELE REGARDING WITH THE SURGICAL TREATMENT

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(Presented by: Naim Hannaoui, Dr.)

Objective: To determine sperm quality in patients diagnosed with varicocele before and after the surgical procedure.

Design and Methods: 60 infertile patients with varicocele (15 clinical varicocele, 19 clinical varicocele after surgical treatment; 16 subclinical varicocele and 10 subclinical varicocele after surgical treatment). Varicocelectomy by Buntz method were performed in 29 of the patients. For sperm quality assessment, computer assisted sperm analysis were done. Data were analyzed using SPSS statistical software package.
Results: Before varicocelectomy, no statistical differences were observed between clinical and subclinical varicocele groups for any of the parameters analyzed. Statistical significant differences were mainly observed among subclinical varicocele after surgical treatment compared to the other groups. Specially, the subclinical varicocele group gets the lowest values for sperm concentration and total sperm number after treatment ($p<0.01$ and $p<0.05$, respectively). Moreover, the type b mobility was also diminished versus the clinical varicocele group after treatment ($p<0.05$), while type d mobility were statistically increased compared with the treated clinical varicocele group ($p<0.05$).
Conclusions: Infertile patients with clinical and subclinical varicocele have a similar sperm quality but varicocelectomy should only be indicated in the clinical group.

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ANTIOXIDANT TREATMENT (ANDROFERTI) IMPROVE DNA SPERM QUALITY IN ASTHENOTERATOZOOSPERMIC INFERTILE MALES

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(Presented by: Carlos Abad, Dr.)

Introduction: Different antioxidants have been used to treat male infertility with different reported results. However, only few studies have been included in their analysis other parameters different from the ones in the spermiogram.
Objective: To evaluate the efficacy of antioxidant treatment (Androferti, Q–Pharma) on sperm quality, DNA integrity and conventional sperm parameters, in patients diagnosed with asthenoteratozoospermia were performed.
Design and Methods: 20 subjects, diagnosed with asthenotheratozoospermia by at least two consecutive sperm analysis, were included in the study. The patients were treated with a commercial oral antioxidant for three months. Sperm parameters, and DNA fragmentation, using Halosperm test, were conducted before and after treatment. For sperm quality assessment, computer assisted sperm analysis were done. Data were analyzed using SPSS statistical software package.
Results: Androferti treatment was able to improve the sperm quality, specially, the vitality ($p<0.01$), progressive motility ($p<0.05$) and DNA fragmentation ($p<0.01$).
Conclusions: Oral antioxidants treatment makes sperm DNA more resistant to fragmentation. These results suggest that antioxidant treatment improves sperm quality not only in seminal parameters but the DNA integrity is also improved. The previous administration of antioxidants could help achieve better outcomes in assisted reproductive techniques.

abstracts full text

SPERMATOGENESIS / STEROIDOGENESIS / TESTIS BIOLOGY

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COMPARISON OF STREPTOZOTOCIN-INDUCED DIABETIC AND INSULIN RESISTANT EFFECTS ON SPERMATOGENESIS WITH PROLIFERATIVE CELL NUCLEAR ANTIGEN IMMUNOSTAINING OF ADULT RAT TESTIS

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(Presented by: Adesina Arikawe BSc; MSc; MBBS; PhD (in view))

Introduction: The overall reproductive processes are well-defined, but the underlying molecular basis of each step, from the formation of germ cells and haploid gametes to the fertilization process, is less understood.
Methods: Adult male Sprague-Dawley rats (120 – 140gm) were randomly divided into 7 groups. Group 1 > control group; fed on normal rat pellets. Group 2 > Type 1 diabetic untreated group; received a single IP injection of streptozotocin 45 mg/kg BW (Guneli et al. 2010) in Na+ citrate buffer pH 4.5. Group 3 > Type 1 diabetic treated group; received IP streptozotocin as in group 2; treated with 0.5 – 1IU isophane insulin. Group 4 > Type I diabetic treated group; received IP streptozotocin as in group 2; treated with 500mg/kg oral ginger daily. Group 5 > insulin resistant diabetic untreated group; fed ad libitum on a special diet containing 25% fructose weight/ weight. Group 6 > insulin resistant diabetic treated group; fed ad libitum on special diet as in Group 5; treated with 15mg/kg oral Pioglitazone daily. Group 7 > insulin resistant diabetic treated group; fed ad libitum on special diet as in Group 5; treated with 500mg/kg oral ginger daily.
Results: Following hyperglycaemia confirmation, animals were perfused with 4% Paraformaldehyde (PFA). Testes were isolated, weighed and fixed in 4% PFA and embedded in paraffin. 5µm thick sections were made and mounted on poly-L-lysine coated slides. Immunohistochemistry was done using PCNA and spermatogenesis was studied at Stage VII (middle) of the spermatogenic cycle through light microscopy.
Conclusion: Mean seminiferous tubular diameter, PCNA index & numerical density were significantly lower ($P<0.05$) in all the experimental groups compared to the control group. Streptozotocin-induced diabetic and insulin resistance impair meiotic division of both primary and secondary Spermatocytes into early spermatids. Germ cells proliferation rate is enhanced by insulin, pioglitazone and ginger administrations.

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RHOX HOMEOBOX GENES ESSENTIAL FOR GERM CELL PROGRESSION IN VIVO

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(Presented by: Miles Wilkinson, PhD)

The X-linked Rhox gene cluster encodes homeobox transcription factors selectively expressed in reproductive tissues. The founding member of the mouse Rhox gene cluster, Rhox5, is highly expressed in Sertoli cells, where it promotes the survival of the adjacent germ cells and promotes male fertility. The in vivo functions of the other 32 genes in the mouse Rhox cluster have not been elucidated. Here, we provide evidence that Rhox3a to Rhox3h—a set of highly related Rhox genes encoding proteins with >96% sequence identity—are essential for spermatogenesis. Because these eight Rhox3 paralogs are interspersed with other Rhox genes, a knockout strategy to determine their in vivo function was not feasible, so instead we used a conditional RNA interference (RNAi) approach. Towards this end, we first identified a short interfering RNA (siRNA) displaying 100% sequence complementary with all eight Rhox3 paralogs that efficiently downregulated RHOX3 in cell lines. This siRNA sequence was inserted (in the form of a short hairpin [sh]) downstream of a ubiquitously expressed Pol III promoter rendered non-functional by a DNA insertion flanked by loxP sites. Several transgenic lines harboring this conditional Rhox3 shRNA construct were obtained and mated with transgenic mice lines expressing Cre recombinase to remove the insert and activate Rhox3 shRNA expression. When mated with mice expressing Cre from a male germ-cell promoter (Stra8), RHOX3 protein level in the testis was reduced by ~50 to ~90%, depending on the Rhox3 shRNA line. Transgenic mice with the strongest reduction in RHOX3 expression had a strong block in post-meiotic germ cell progression and >1% of normal sperm count. These Rhox3-knockdown mice also had reduced numbers of spermatocytes, suggesting they also a partial block in male meiosis. Using microarray analysis, we identified many genes misregulated in Rhox3-knockdown mice testes. We are currently conducting experiments to determine which of these are direct target genes. To our knowledge, the Rhox3 paralogs are the first members of the Rhox gene cluster shown to have a direct role in germ cells. We propose that the conditional RNAi approach that we used to address their in vivo functional role has the potential to be a generally applicable and efficient means to elucidate the cell type-specific functions of genes.

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OXIDATIVE STRESS MEDIATED UPREGULATION OF MITOCHANDRIAL DEPENDEND GERM CELLAPOPTOSIS IN STREPTOZOTOCIN INDUCED DIABETIC MALE RAT

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(Presented by: Sekar Suresh, PhD)

The objective of present study is to analyze role of hyperglycemia on apoptotic dysregulation and germ cell apoptosis in long-term hyperglycemic rat testis. Wister albino rats (Rattus norvagicus) were randomly divided into two groups i.e. Control (received 0. IM of citrate buffer) and diabetes (streptozotocin at 60 mg/kg in 0.01M citrate buffer through i.p.). After 16 weeks animals were sacrificed by overdose of anesthesia (thiopentone sodium 60 mg/kg i.p.). testicular tissue were subjected to estimation of ROS and RNS production, Daily sperm production (DSP), immunoblotting, immunohistological and qPCR (BCl2, Bax, Bcl-XL, caspase3 and cytochrome-C). Apoptosis analyses were done by TUNEL stain. Results showed significant increases in production of ROS ($p<0.001$), RNS (0.01) and LPO (0.001) 16 week STZ injected animals. The DSP significantly (0.001) reduced which was significantly associated with the increased apoptosis positive (0.01) cells was observed in STZ animal. Histological study showed severe degenerative changes in seminiferous epithelium at

16 week STZ animal when compared to control. In immunohistochemistry and immunoblotting study, expression of the Bax, cytochrome-C and caspase 3 were increased associated with diminished expression of levels of Bcl2, Bcl-XL, in the 16 week STZ injected animals. Present data clearly demonstrates that prolonged hyperglycemia up-regulate oligomarization and translocation of Bax to mitochondria leading to release of cytochrome C and activation of the caspase cascade resulted in germ cell apoptosis. Further understanding of the hyperglycemia induced germ cell apoptosis may allow new targeted approaches to treat diabetic dependent male infertility.

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MOUSE RC/BTB2, A MEMBER OF THE RCC1 SUPERFAMILY, LOCALIZES TO THE ACROSOMAL VESICLE THROUGH ITS BTB DOMAIN IN ROUND SPERMATIDS

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(Presented by: Maria Eugenia Teves, PhD)

Mouse chromosome condensation 1-like (BC003224), now called RC/BTB2, is an unstudied protein of the RCC1 (Regulator of Chromosome Condensation) superfamily. Because of the significant remodeling of chromatin that occurs during spermiogenesis, we characterized the expression and localization of mouse RC/BTB2 in the testis and male germ cells. The Rc/btb2 gene yields two major transcripts: 2.3 kb Rc/btb2-s, present in most somatic tissues examined; and 2.5 kb Rc/btb2-t, which contains a novel non-translated exon in its 5'-UTR, and is only detected in the testis. During the first wave of spermatogenesis, Rc/btb2-t mRNA is expressed from day 8 after birth, reaching highest levels of expression at day 30 after birth. The full length protein contains three RCC1 domains in the N-terminus, and a BTB domain in the C-terminus. In the testis, the protein is detectable from day 12, but is progressively up-regulated to day 30 and day 42 after birth. In spermatids, the protein is mainly localized to the acrosomal vesicle capping the nuclei in a position opposite to the manchette, a structure involved in reshaping the sperm head and forming the sperm tail. RC/BTB2 co-localizes with components of the acrosome in immuno-gold EM studies, and thus appears to mark the site of acrosome biogenesis. A GFP-tagged RCC1 domain is present throughout the cytoplasm of transfected cells. However, both GFP-tagged full length RC/BTB2 and a GFP-tagged BTB domain localize to the Golgi-like region, closely associated with the nuclear membrane, suggesting that the BTB domain mediates protein targeting. Since RCC1 domains associate with Ran, a small GTPase that regulates molecular trafficking, RC/BTB2 might play a role in compartmentalization in the the developing acrosome, and possibly its positioning on the spermatid nuclear membrane.

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TESTIS VOLUME AND THE PRESENCE OF SPERM IN THE EJACULATE OF ADOLESCENTS WITH KLINEFELTER SYNDROME

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(Presented by: Akanksha Mehta, MD)

Introduction: The use of fluorescent microscopy for the detection of rare sperm in the ejaculate of adolescents with Klinefelter syndrome has been described in a limited number of patients, but the relationship, if any, between the presence of sperm and patient age or testis size has not been previously investigated. The objective of this study was to update previous results on sperm detection using florescent microscopy in a larger cohort of patients, and to analyze the chance of sperm detection with respect to age and testicular volume measured by ultrasonography (US).
Methods: The study population consisted of adolescents with KS treated at our institution. All patients underwent history and physical examination, serum hormone analyses, and scrotal US as per our standard evaluation. An ejaculated semen sample was obtained from patients who were able and willing to masturbate. Semen samples were centrifuged. The high density pellet was extracted within 10uL of solution, spread across a glass slide, fixed, permeabilized, and dried. The slides were stained with ProLong Gold antifade reagent with DAPI (Invitrogen) and analyzed using a Nikon Eclipse 50i fluorescent microscope equipped with an ultraviolet fluorescence filter and a digital camera. Sperm were identified by their characteristic size and morphology. Testis size was measured by scrotal US performed by the same examiner in each patient. Testis volume was calculated using the formula V=0.72*l *w*h, and averaged between the two testicles.
Results: 59 sequential patients with KS who underwent semen analyses and scrotal US were enrolled in the study. Mean patient age was 15.3 years. Mean serum FSH was 26.9 ± 20.6 IU/L (range 0.05-74.38 IU/L). Overall, sperm were found in the ejaculate in 41/59 (69.5%) of boys. There was no statistically significant difference in age (15.2 vs. 15.5 years) or testicular volume (2.8 vs. 3.0 mL) between patients who had sperm found versus those who did not. There was a trend towards an increased chance of finding sperm in boys aged 15-16 years of age, compared to younger or older boys.
Conclusion: Ejaculated sperm were present in approximately 70% of adolescent boys with KS using fluorescent microscopy, which is an improvement over previously reported data. There was a trend towards increased chance of sperm detection in boys aged 15-16 years of age. No significant correlation could be found between testis volume and the presence of ejaculated sperm in this study.

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CHARACTERIZATION OF A NOVEL TESTIS-SPECIFIC PROTEIN LOCALIZED TO THE GOLGI/ACROSOME OF POST-MEIOTIC GERM CELLS

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Oklahoma Medical Research Foundation
(Presented by: Edward Nguyen)

Our laboratory performed a screen to identify putative tyrosine-sulfated proteins expressed in the male genital tract using anti-sulfotyrosine antibody affinity chromatography coupled with mass spectrometry. In this

screen a novel protein enriched from the soluble fraction of mouse epididymis was identified. Review of the public genomic and expression databases indicate that the gene spans 8 exons on chromosome 8 and the protein is expressed from a 761 bp transcript that is detected only in testis. The transcript predicts a 182-residue precursor with a 24-residue signal peptide, a single potential N-glycosylation site and no cysteine residues and thus should be secreted as a 17.3 kDa monomer assuming that no post-translational modifications are present. Orthologous genes are present in the rat, rabbit, and various primates including man. However, the protein has no homology to any known protein and therefore we have little insight into its function(s). In order to begin exploring the function of this protein we first examined when and where the protein was expressed. To do so, we developed a rabbit antibody using purified bacterial recombinant 158-residue mature polypeptide as the immunogen.

Western blots of adult testis detects a single protein of ≈20 kDa that does not change in electrophoretic mobility in the presence of reducing agents or after digestion with peptide:N-glycosidase F. Subcellular fractionation of testis indicates that the protein is primarily in the membrane fraction, but a small percentage is also detectable in the soluble fraction. Western blotting detects the protein only in the testis beginning at ≈ P21 coinciding with the onset of spermiogenesis but not in epididymis or 10 other tissues examined. We next examined what cells express the protein by immunofluorescence staining of tissue sections and cytologic smears of germ cells isolated from seminiferous tubules. Our analyses show that the protein is expressed only in post-meiotic germ cells beginning at the round spermatid stage. The staining pattern in round spermatids is primarily a bright peri-nuclear spot reminiscent of Golgi, morphing into an acrosomal staining pattern in elongating spermatids and spermatozoa. The staining also co-localizes with Syntaxin6, a marker of the Golgi. In summary, we have identified a novel protein localized to the Golgi/acrosome of post-meiotic germ cells. Our studies provide a foundation to begin exploring the function of this interesting protein.

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HUMANIN PREVENTS HEAT-INDUCED GERM CELL APOPTOSIS VIA ITS COGNATE RECEPTOR IN AN EX VIVO SEMINIFEROUS TUBULE CULTURE SYSTEM

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(Presented by: Yue Jia MD, PhD)

Objective: Humanin (HN) has been identified as an endogenous cytoprotective peptide. HN protects neuronal cell from death and dysfunction by interacting with a cytokine receptor complex on the cell surface involving the IL-27 receptor subunit: WSX-1, ciliary neurotropic factor α (CNTFRα), and gp130 (the common subunit of IL-6 receptor family). Using an ex vivo seminiferous tubule culture system, we evaluated whether HN prevents heat-induced male germ cell apoptosis and whether the mechanism of action involves the putative receptor.
Methods: Mouse seminiferous tubules were micro-dissected and light stages (early and late) were isolated and processed in serum free media. Dissected seminiferous tubules were heated at 43 C for 30 minutes (or kept in 37 C for the control group) before culture. After heating, HN or scrambled peptides were added to the media.
To study the possible mechanism of HN, anti-WSX-1, anti-CNTFRalpha, or anti-GP130 antibody was added into the culture media (1 mcg/ml) 15 minutes before the HN peptide, and non-immune IgG was used as negative control for each specific antibody. Seminiferous tubules were incubated at 34 C with 5% CO2 for 15 hours and then collected. Apoptotic cells were quantified by flow-cytometry with combined Annexin V conjugated with allophycocyanin and 7-Amino Actinomycin D, which was used to label apoptotic and non-viable cells, respectively.
Results: Heat-induced male germ cell apoptosis was prevented by co-incubation with HN peptide but not scrambled peptide. Anti-WSX-1 and anti-GP130 antibodies significantly blocked the protective effect of HN peptide on apoptosis ($p<0.05$, compared with normal IgG treatment) in this ex vivo model although the anti-CNTFRalpha was not effective in this regard.
Conclusions: HN prevents heat-induced male germ cell apoptosis in an ex vivo mouse seminiferous tubules culture. Two of the cytokine receptor subunits, WSX-1 and GP130, are involved in the protective effect of HN in seminiferous tubules. CNTFR alpha, which is involved in the HN protective effect in neuronal cell may not play an important role in HN related effects in testis. These findings suggest that the cytoprotective effect of HN may involve different receptor(s) in different tissues such as brain and testis. These observations will be useful in developing tissue-targeted therapies.

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ACTIVIN-HORMONE CROSSTALK IN TESTIS DEVELOPMENT AND DYSGENESIS: CLUES FROM INHIBIN ALPHA HETEROZYGOUS AND KNOCKOUT MICE

Catherine Itman, PhD and Kate Loveland, PhD
Monash University
(Presented by: Catherine Itman, PhD)

Introduction: Activin A, a dimeric cytokine formed from inhibin βA subunits, regulates testis development and fertility by acting locally on testicular cells and distally, promoting pituitary production of follicle stimulating hormone. Inhibin, a heteromeric inhibin α/inhibin β dimer, antagonizes activin production and activity. Male mice lacking inhibin develop Sertoli cell tumors, identifying inhibin α as a tumor-suppressor. Pituitary gonadotropins, androgens and estrogens contribute to Sertoli cell tumors, yet how hormones and activin interact to promote tumorigenesisis not understood.
Objective: To identify the mechanism/s by which activin influences hormone action during testis development and dysgenesis.
Methods: Histological and immunohistochemical assessment of Sertoli cell proliferation and maturation were performed using testis sections from Inha+/+, Inha+/- and Inha-/- mice at 7 ($n\geq3$ per genotype), 16 ($n\geq6$ per genotype) and 28 days of age ($n\geq6$ per genotype).
Results: Sertoli cells of 16 and 28 day Inha-/- mice exhibited features of immaturity, with intense Anti-Mullerian Hormone immunoreactivity, mislocalized Connexin43 (blood-testis barrier protein) and ongoing proliferation. Sertoli cells had detached from the basement membrane and were clustered in the lumen. Predominantly cytoplasmic Androgen Receptor (AR) localization indicated impaired androgen signaling. Unexpectedly, juvenile (16 and 28 day) Inha+/- mice also had a testicular phenotype, despite being fertile and apparently healthy as adults. Some tubules displayed advanced development, with round spermatids present at 16 dpp, coinciding with apparently advanced Sertoli cell maturation. Other tubules contained dysgenic regions with some features of immaturity similar to Inha-/- testes, yet there were distinct differences: no proliferating Sertoli cells were detected and no Sertoli cell clusters were found in the lumen.

Intriguingly, Sertoli cells in dysgenic regions also had reduced AR nuclear localization but apparently increased inhibin α subunit production relative to adjacent normal tubules.
Conclusion: Distinctly different testicular phenotypes of Inha−/− and Inha+/− mice identify important, dose−dependent roles for activin in testis development, dysgenesis and tumorigenesis. In particular, evidence of impaired androgen signaling in aberrant Inha+/− and Inha−/− Sertoli cells highlight a critical role for regulated activin production and action in mediating androgen signaling.

Monday, April 23, 2012
11:00 a.m. – 12:30 p.m.

Poster Session II
Location: Exhibit Hall

ANDROGENS / ENDOCRINOLOGY

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MALE HYPOGONADISM IN A FEMALE TRANSSEXUAL

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GWUH
(Presented by: Shant Ayanian, MD)

Introduction: A 29 year old male−to−female transsexual presented for consultation regarding hormonal management. Baseline hormonal testing revealed a profoundly low total testosterone in the male hypogonadal range (80 ng/dl), a total estradiol at the upper end of normal (34 pg/ml), and a normal FSH and LH (1.8 and 4.1 mIU/ml, respectively). Further lab testing showed a low sex hormone binding globulin (14 nmol/L), DHEA−S (75 μg/dL) and inhibin B (49.5 pg/mL). Other studies showed a normal pituitary MRI, a negative Angelman/Prader Willi assay and a normal 46XY chromosomal analysis. The physical exam was remarkable for superobesity (BMI of 58), small testicular volumes of 6 and 8 ml and possible gynecomastia.
Methods: To elucidate estradiol's role in the regulation of the hypothalamic−pituitary−gonadal axis, the patient took an aromatase inhibitor (letrozole 2.5 mg orally twice daily) for 14 days.
Results: Treatment with an aromatase inhibitor reduced the estradiol to 1.9 pg/ml with a dramatic increase in total testosterone to 309 ng/dl which was accompanied by increased levels of FSH and LH (15.3 and 20.5mIU/ml, respectively).
Conclusions: Ironically, this case involves a male−to−female transsexual who already had partial success in lowering her androgens and raising her estrogens without hormonal therapy or surgery. The use of an aromatase inhibitor as a diagnostic test confirmed the potent role of estradiol in the negative feedback on the hypothalamic−pituitary−gonadal axis. This case is consistent with the inverse association between superobesity and hypogonadal testosterone levels, possibly due to estradiol from increased aromatization. The etiology of the small testicular volumes is unclear and cannot be explained by the obesity alone. The patient will begin estradiol treatment to promote her physical transition to a woman. This case represents a unique combination of gender identity disorder and male hypogonadism.

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MEDICALLY ALTERING THE TIMING OF RUT IN MALE PACIFIC WALRUS (ODOBENUS ROSMARUS DIVERGENS) TO ACHIEVE A PREGNANCY IN A NULLIPAROUS FEMALE

Holley Muraco, BS[1], Leah Coombs[1], Diana Procter[1], Paul Turek, MD[2] and Michael Muraco[1]
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(Presented by: Holley Muraco, BS)

Introduction and Objectives: The reproductive rate of Walrus in United States zoos is extemely low reproductive at 11 births in 80 years. This is partly due to the fact that little is known about Pacific walrus reproductive biology. To address this lack of knowledge, we initiated a 7−year study in which detailed biological data were recorded on captive walrus. The goal was to better understand the relative timing of the male rut and female ovulation.
Methods: In a zoological institution, one male and one female 16−year−old captive Pacific walrus were carefully monitored with weekly serum hormone analysis, daily glans penis smears for sperm, and abdominal ultrasound for pregnancy. Based on these observations, hormonal manipulation of the male rut was undertaken to improve the pregnancy rate.
Results: We observed that the female ovulated once annually from late December through mid−January, afterward exhibiting 9 months of sustained elevated progesterone. In contrast, the male's seasonal rut routinely occurred asynchronously in late February through May with a serum testosterone peak in March. During the female's ovulation, the male had nadir testosterone levels and was consistently azoospermic. Likewise, during the male's spermatogenic rut, the female was anovulatory with elevated progesterone. Based on this, the male was treated with 14−weeks of human chorionic gonadotropin (hCG) to increase testosterone levels in synchrony with the female's annual ovulation. The treatment successfully induced rut and the pair successfully bred, and the female became pregnant. Upon discontinuation of hCG treatment, the male resumed baseline testosterone levels. The timing and response to hCG in the treatment cycle that resulted in pregnancy is illustrated in the figure.
Conclusion: We theorize that the lack of synchronization of rut and ovulatory cycles is a primary reason for the reproductive failure in these captive walrus. We suspect that this occurs due to displacement of the species from its natural artic habitat and photoperiod cues. This information may inform reproductive programs worldwide for this animal species.

ENVIRONMENT / TOXICOLOGY

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RANDOMIZED CONTROL DIETARY TRIAL OF WALNUTS ADDED TO WESTERN STYLE DIET: IMPACT ON SERUM FATTY ACID PROFILES, SPERM VITALITY, MOTILITY, AND MORPHOLOGY

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(Presented by: Wendie A. Robbins, PhD)

Introduction: Polyunsaturated fatty acids (PUFAs), essential to male reproduction, modulate key enzyme pathways in steroid metabolism and provide fluidity to the sperm plasma membrane needed for motility and oocyte fusion.

Levels of PUFAs change throughout spermatogenesis being three to six fold higher in testicular compared to epididymal or mature germ cells. Predominant sperm PUFAs are arachidonic (AA), docosahexaenoic (DHA), and linoleic (LA). Although essential, excessive levels of PUFAs may subject sperm to oxidative stress (AA, DHA, and finally LA in order of reactivity). Damage would depend on an individual's anti-oxidant capacity, for example, anti-oxidants present in seminal plasma.

Objective: The objective of this research was to determine if enriching a Western style diet with the plant source of omega-3, α-linolenic acid (ALA), in walnuts would improve FA profiles in blood serum and sperm and improve semen quality. Walnuts as a whole food provide essential omega-3 as well as important anti-oxidants, for example selenium.

Methods: In a randomized control trial involving 117 healthy men ages 20 – 35 eating Western style diets, participants ate usual diet supplemented with 75 gm whole-shelled walnuts per day or continued usual diet avoiding tree nuts for three months. Baseline and follow-up measures included weight, height, BMI, exercise pattern (IPAQ), semen analysis (WHO 1999), blood serum and sperm membrane fatty acid (FA), seminal fluid anti-oxidant capacity, blood selenium, zinc, folate and hormones. Every two weeks participants completed a 24-hour dietary recall using ASA24 (National Cancer Institute) software for nutrient analysis.

Results: Baseline FA analysis for the study population was consistent with published literature for blood serum or plasma with highest to lowest percent of total FA being LA, Palmitic, Oleic, Stearic, AA, DHA, ALA; and in sperm membrane pilot data, highest to lowest were Palmitic, DHA, Stearic, Oleic, LA, AA, ALA. Compared to controls, men in the walnut intervention showed improved sperm vitality p=0.003, motility p=0.04, and morphology p=0.04 with increased serum omega-3 ALA p=0.0001, while the more oxygen reactive omega-3, DHA, did not differ with the intervention.

Conclusions: Walnuts provide a balanced, whole food approach to deliver PUFAs plus anti-oxidants essential for male fertility.

Funding: This work was funded by the California Walnut Commission and UCLA Center for Occupational and Environmental Health.

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CYCLOPHOSPHAMIDE TREATMENT AFFECTS MICRORNA EXPRESSION PROFILES IN MALE RAT GERM CELLS

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(Presented by: Anne Marie Downey, MSc)

Introduction: MicroRNA's are a class of small, non-coding RNA molecules involved in post-transcriptional regulation of gene expression. They act by complementary base pairing with the 3' un-translated region of a target messenger RNA causing either its degradation or translational repression, depending on the degree of complimentarity. Studies suggest that miRNA's likely play a role in regulating gene expression throughout spermatogenesis. Previous studies in our laboratory have shown that paternal exposure to cyclophosphamide, a chemotherapeutic and immunosuppressant, has an effect on gene expression and protein content in male rat germ cells. The mechanism by which these changes occur is not known. The goal of this study is to test whether cyclophosphamide will affect miRNA expression profiles in male rat germ cells.

Methods: Male rats were treated orally with either cyclophosphamide or saline 6 days a week for 4 weeks. Round spermatids from both control and treated rats were collected by unit gravity sedimentation using the STA-PUT method. Total RNA (including miRNA) was isolated and verified for integrity and presence of miRNA. miRNA and mRNA expression was profiled using a whole rat genome miRNA and gene expression microarrays. Data was analyzed with Genespring Software 11.5.

Results: Analysis of preliminary miRNA microarray data show that samples from treated and control animals have differential miRNA expression profiles. 182 miRNA's showed over two-fold change in expression in treated samples compared to control. 51 of these were up-regulated while 131 miRNAs were down-regulated. A target scan of up-regulated and down-regulated miRNA's revealed that these miRNA's potentially regulate many genes. Gene ontology analysis of the targets up and down regulated miRNA's revealed that target genes are involved in a multitude of processes. Gene expression microarray data show differential mRNA expression profiles between treated and control samples. Some of these mRNA's are predicted targets of the up and down regulated miRNA's.

Conclusion: Preliminary data suggests that cyclophosphamide may have an effect on miRNA expression profiles. Up and down regulated miRNA's potentially target genes that are involved in a multitude of processes. Many of these potential targets also show changes in expression. This suggests that changes in miRNA expression profiles in round spermatids could potentially have many consequences on the proper development of male gametes.

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EFFECTS OF PHTHALATES ON 3β-HYDROXYSTEROID DEHYDROGENASE AND 17β-HYDROXYSTEROID DEHYDROGENASE 3 ACTIVITIES IN HUMAN AND RAT TESTES

Ying Su, MD[1], Binghai Zhao, PhD[2], Ren-Shan Ge, MD[3] and Qing-Quan Lian, PhD[1]

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(Presented by: Ying Su, MD)

Introduction and Objectives: The 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) are involved in the reactions that culminate in androgen biosynthesis in Leydig cells. Human and rat testis microsomes were used to investigate the direct inhibitory potencies of 11 phthalates with various carbon numbers in the ethanol moiety on 3β-HSD and 17β-HSD3 activities.

Methods: The conversions of pregnenolone into progesterone for 3β-HSD activity and androstenedione into testosterone for 17β-HSD3 activity in human and rat testis microsomes were measured by radiolabeled substrate, chromatographic separation and radiometric scanning analysis.

Results: The results demonstrated that the half maximal inhibitory concentrations (IC50s) of dipropyl (DPrP), dibutyl (DBP), dipentyl (DPP), bis(2-butoxyethyl) (BBOP) and dicyclohexyl (DCHP) phthalate were 123.0, 24.1. 25.5, 50.3 and 25.5 μM for human 3β-HSD activity, as well as 62.7, 30.3. 33.8, 82.6 and 24.7 μM for rat 3β-HSD activity, respectively. However, only BBOP and DCHP potently inhibited human (IC50s, 23.3 and 8.2 μM) and rat (IC50s, 30.24 and 9.1 μM) 17β-HSD3 activity. Phthalates with one to two or seven and eight carbon atoms in ethanol moieties had no effects on both enzyme activities even at concentrations up to 1 mM.

The mode of action of DCHP on 3β–HSD activity was competitive with the substrate pregnenolone but noncompetitive with the cofactor NAD+. The mode of action of DCHP on 17β–HSD3 activity was competitive with the substrate androstenedione but noncompetitive with the cofactor NADPH.

Conclusion: In summary, our results showed that there is clear structure–activity responses for phthalates in the inhibition of both 3β–HSD and 17β–HSD3 activities. The length of carbon chains in the ethanol moieties of phthalates may determine the potency to inhibit these two enzymes.

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DIRECT INHIBITORS OF TESTICULAR 17α-HYDROXYLASE ACTIVITY

Leping Ye, MD[1], Ren-Shan Ge, MD[2] and Qingquan Lian, MD[1]
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(Presented by: Leping Ye MD)

Introduction and Objectives: Many endocrine disruptors are antiandrogens. Some may act via directly inhibiting steroidogenic enzymes in Leydig cells. The objectives of the study are to screen several categories of endocrine disruptors for the inhibition of CYP17A1 17α–hydroxylase.

Methods: CYP17A1 17α–hydroxylase activity was measured by the conversion of 3H–labeled progesterone into 17α–hydroxyprogesterone in the presence of NADPH and human and rat testicular microsomes with/without 250 μM inhibitors. Six categories of endocrine disruptors were screened, including drug (ketoconazole), plasticizers (12 phthalates, bisphenol A), plant constituents (gossypol and genistein), surfactants (4 perfluoroalkyl substances), smoke constituents (nicotine) and insecticides (methoxychlor).

Results: Phthalates (from dimethyl phthalates to di diisononyl phthalate), perfluoroalkyl substances (including PFOS and PFOA), genistein and nicotine did not inhibit human 17α–hydroxylase activity. Ketoconazole, gossypol, bisphenol A and methoxychlor metabolite HPTE inhibited human 17α–hydroxylase activity with Ki of 293, 30280, 3342, and 532 nM. These chemicals had the similar inhibitory effects on rat 17α–hydroxylase activity. Ketoconazole and bisphenol A and HPTE are the competitive inhibitors of 17α–hydroxylase activity.

Conclusion: We identified several potent environmental inhibitors for 17α–hydroxylase activity, including HPTE and gossypol.

EPIDIDYMIS / VAS DEFERENS / SEMINAL VESICLES

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LOCALIZATION OF SPAG11C IN EMBRYONIC AND POSTNATAL EPIDIDYMIS UNRAVELS ANDROGEN-DEPENDENT MECHANISMS REGULATING ITS EXPRESSION

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(Presented by: Camilla Ribeiro Master)

Sperm–associated antigen 11 (Spag11) is a beta–defensin–like gene expressed in the male reproductive tract. Although Spag11 gene mRNA expression is known to be regulated by androgens, the mechanisms underlying this modulation remain unclear. SPAG11C, one of the several isoforms encoded by this gene, is abundantly expressed in the epididymal epithelia, is found in association with epididymal and ejaculated sperm and the recombinant protein acts in vitro as an antibacterial agent. Here, we determined SPAG11C spatiotemporal expression (protein and mRNA) over the life span of the Wistar rat, focusing on the development of the epididymis, in order to gain insights into the regulation and potential physiological roles of this specific SPAG11 gene isoform. Whole embryos were collected at embryonic days 14, 16, 18 and 20 and epididymides isolated from 1, 5, 10, 20, 40 and 120–day–old rats. Spatiotemporal SPAG11C expression was evaluated by immunohistochemistry and in situ hybridization. Prenatally, SPAG11C was widely distributed in sites of different embryonic origins, including reproductive tract and extragenital tissues at all time–points analyzed. Positive staining was observed in the testis, Wolffian duct, kidney, muscle (smooth, skeletal and cardiac), chondrocytes, adrenal gland, lung, liver, pancreas and in the nervous system. In situ hybridization revealed the presence of Spag11c mRNA in the same sites immunopositive for SPAG11C. Thus, the widespread expression of SPAG11C in embryo broadens the potential biological roles of this protein within and beyond the male reproductive tract. We then compared the SPAG11C distribution pattern with that of the androgen receptor (AR) in prenatal and postnatal epididymis. Prenatally, both SPAG11C and AR immunoreaction were predominantly distributed in the mesenchymal cells of the Wolffian duct, specially surrounding the duct epithelium. Following birth, however, the predominant localization of SPAG11C and AR immunostaining switched from embryonic mesenchymal cells to epithelial cells of the epididymis. The overlapping distribution of these two proteins suggests that Spag11 gene is a target for direct effects of androgens in epididymis and in its anlagen, the Wolffian duct. Additionally, we hypothesize that SPAG11C may be involved in cell growth/differentiation as well as in epithelial–mesenchymal or epithelial–stromal signaling in the Wolffian/epididymal duct.

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CIRCULATING ALDOSTERONE INDUCES THE APICAL ACCUMULATION OF THE PROTON PUMPING V-ATPASE IN CLEAR CELLS IN THE CAPUT EPIDIDYMIS

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(Presented by: Jeremy Roy, PhD)

A major function of clear cells located in the epithelial layer of the epididymis is to facilitate luminal acidification. Clear cells perform this function by pumping H+ ions into the lumen via the V–type H+–ATPase localized to microvilli projections emanating from their apical membrane. Acidification of the epididymal lumen, and thus clear cell activity, is important for keeping spermatozoa in a quiescent state and therefore male fertility. We have shown previously that clear cell activity is regulated by numerous factors including, members of the renin–angiotensin system (RAS). A downstream effector of RAS is the production of the mineralocorticoid aldosterone, which is released in response to angiotensin II. Previous studies showed binding of aldosterone to clear cells specifically (Hinton and Keefer, 1985). Here we show that the mRNA expression of both the mineralocorticoid receptor (MR) and 11–β–dehydrogenase isozyme 2 (HSD11β2) is enriched in clear cells, further suggesting that these cells are poised to respond to the action of aldosterone. We then investigated the non–genomic signaling pathway utilized by aldosterone in clear cells. Sprague–dawley rats were injected via the dorsal tail vein with a bolus of aldosterone (200 ul; 200 nM) followed by infusion over a 15 min period (600 ul; 200 nM).

Epididymides were fixed, cryosectioned and labeled for the V-ATPase B1 subunit and the apical marker, clathrin. The length of the V-ATPase-labeled microvilli domain was measured as an assessment of clear cell activation. Aldosterone caused a region specific elongation of clear cell microvilli. Aldosterone increased the average microvilli domain length by 48% ($P < 0.05$) in the caput, but had no statistically significant effect in other regions, including the cauda. Elongation of the V-ATPase-labeled microvilli domain was also induced following tail vein injection of 1,2-dioctanoyl-sn-glycerol (DOG; 20 uM), a cell-permeant diacylglycerol (DAG) analog, and cpt-cAMP (20 uM), a cell-permeant analogue of cAMP, implicating both pathways in the non-genomic regulation of caput epididymal clear cells. In conclusion, our results show that clear cells in the caput region of the epididymis respond to circulating levels of aldosterone, thus solidifying the renin-angiotensin-aldosterone system (RAAS) in the regulation of male fertility. This study was funded by NIH grant HD040793.

FERTILIZATION / GERM CELL DIFFERENTIATION / REPRODUCTIVE DEVELOPMENT

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TESTIS-SPECIFIC SERINE/THREONINE KINASE (TSSK) FUNCTION IS NECESSARY FOR SPERMIOGENESIS IN CAENORHABDITIS ELEGANS

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(Presented by: Matthew Marcello, PhD)

Testis-specific serine/threonine kinases (TSSKs) play a crucial role in sperm development and fertilization in mammals. Mice express five active Tssk genes (Tssk1-4 and Tssk6) and Tssk1, Tssk2, and Tssk6 are essential for fertility. Tssk1/2 knockout mice are unable to produce normal spermatozoa, and sperm from Tssk6-null mice are unable to fuse with eggs. The nematode Caenorhabditis elegans expresses three Tssk orthologs: B0511.4, C27D6.11, and Y38H8A.4. We have obtained deletion mutants for all three C. elegans Tssk genes and we are evaluating their function in sperm development and fertilization. In addition to the single mutants, we have generated a B0511.4; Y38H8A.4 double mutant. At this time, we have evaluated the function of B0511.4 and Y38H8A.4 in sperm development and fertilization using the respective single mutants as well as the B0511.4; Y38H8A.4 double mutant. We found that strains lacking either B0511.4 or Y38H8A.4 and the B0511.4; Y38H8A.4 double mutant are able to produce progeny. However, when in vitro spermiogenesis assays were performed, sperm from all three mutant strains were unable to undergo spermiogenesis normally. Additionally, our preliminary analysis indicates that sperm from B0511.4-deficient animals have defects in sperm competition. We are currently working to create a triple mutant strain deficient in all three C. elegans Tssk genes. Using the Tssk triple mutant, along with the single mutants and double mutant pairs, we aim to define the role of Tssk genes in C. elegans sperm development and fertilization. Future experiments will further define the spermiogenesis defect observed in the Tssk mutants and clarify if these defects lead to less competitive sperm or fertility defects.

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ROLE(S) OF MOUSE SPERM ASSOCIATED ALPHA-L-FUCOSIDASE IN FERTILIZATION

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(Presented by: Kamonrat Phopin, PhD)

Introduction and Objectives: Previously, we documented the existence of alpha-L-fucosidase in mouse cauda epididymal contents and showed that sperm-associated α-L-fucosidase is cryptically stored within the acrosomal compartment and following acrosome reaction, becomes predominantly localized within the sperm equatorial segment. Here, we evaluated whether mouse sperm associated α-L-fucosidase is required for early embryogenesis and/or any steps of fertilization.
Methods: Control, DFJ, or anti-fucosidase pretreated capacitated mouse sperm were inseminated into each IVF drop containing oocytes. For some experiments, zona-intact or zona-free oocytes were pretreated with purified fucosidase for 1 h prior to insemination. After gamete co-incubation for various lengths of time at 37 °C, 5% CO2, the oocytes were pipetted up and down 3 times in PBS to dislodge sperm bound loosely to the oocytes. Then, we observed the number of sperm tightly bound or fused to oocytes. In addition, intracytoplasmic sperm injection and incubation of 2-pronuclear (2-PN) embryos with 5 mM DFJ were performed to disclose the roles of sperm associated α-L-fucosidase in post-fusion events and/or early embryogenesis.
Results: However, the specific and potent competitive inhibitor 5 mM DFJ did not significantly inhibit sperm-egg zona, membrane binding, or fusion. Anti-fucosidase antibody and purified human liver fucosidase competed significantly to reduce the number of sperm bound and fused to oocytes. These findings indicate that the fucosidase glycoprotein plays a role in sperm-egg zona, membrane binding, and fusion steps; this interaction is primarily associated with fucosidase structural binding, and not in the catalytic site of α-L-fucosidase. Fusion and penetration by sperm is apparently rate limited by the prior membrane recognition and tight binding steps that involve the fucosidase glycoprotein. Moreover, intracytoplasmic sperm injection showed no significant difference in 2-cell embryo development in the 5 mM DFJ pretreated sperm group compared to the untreated control and 5 mM DFJ did not inhibit embryo development from 2-PN to blastocysts.
Conclusion: The recognition and binding of mouse sperm to the zona pellucida and oolemma involves the glycoprotein structure of α-L-fucosidase, but not its catalytic action. These observations suggest that deficits in fucosidse protein and/or the presence of anti-fucosidase antibody may be responsible for some types of infertility.

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ISOLATED ACROSOMAL MATRICES FROM MOUSE SPERMATOZOA CONTAIN AMYLOID-LIKE STRUCTURES AND CRES (CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC) SUBGROUP MEMBERS

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(Presented by: Benoit Guyonnet, PhD)

Amyloids, highly ordered protein aggregates, are commonly associated with pathologies but have also been shown to have functional roles including as a scaffold for the synthesis of melanin and as storage for pituitary hormones. We have previously shown that the cystatin CRES (Cst8) forms amyloid in vitro and in vivo within the epididymal fluid. CRES is also present in the acrosome suggesting it could contribute to the formation of a functional amyloid-like structure involved in fertilization.

Using conformational antibodies that recognize either the oligomeric or fibrillar forms of amyloid and amyloidophilic fluorophores, we show that amyloid-like structures are present in the acrosomes of testicular and epididymal mouse spermatozoa, and associated specifically with its particulate fraction as shown by the immunostaining of acrosomal shrouds and isolated acrosomal matrices (AM). While both forms of amyloid were detected in the intact acrosome, after acrosome reaction amyloid associated with the acrosomal cap was removed with the cap while a new pattern for oligomeric amyloid, showing a crescent shape overlying the equatorial segment, was observed. Finally, incubation of isolated AM with PAD ligand that binds amyloid, captured CRES supporting that it contributes to the formation of this amyloid-like structure.

Proteomic analysis using LC-MS/MS was carried out to identify proteins in the isolated AM from cauda epididymal spermatozoa. Around 300 unique proteins were identified of which half were not found by previously published proteomic analysis of whole spermatozoa. In AM compare to whole spermatozoa, we found an enrichment of proteins involved in "cellular component morphogenesis"and "structural molecule activity." We identified ten AM markers and several acrosomal proteins specifying their localization to the AM. Several proteins predicted to form amyloid and belonging to family groups with at least one member that is amyloidogenic were identified including the lysozyme family members (Lyz1, LyzL3-4-5 & 6) and the CRES subgroup member cystatin T (Cst13). CRES was identified by Western blot of purified AM.

Taken together, our studies suggest that amyloid-like structures are present within the mouse sperm acrosomal matrix and that several proteins from different families may contribute to their formation. Amyloid-like structures could be involved in several steps of fertilization as they are associated with acrosome intact and acrosome reacted spermatozoa.

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THE CONTRIBUTION OF REGULATED NUCLEOCYTOPLASMIC TRANSPORT TO PARASPECKLE FORMATION AND ITS POTENTIAL ROLE IN TRANSLATIONAL CONTROL OF SPERMATOGENESIS

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(Presented by: Andrew Major, BSci, Honours)

Spermatogenesis requires progressive gene expression changes effected by controlled nuclear access of transcription factors and other proteins. Many proteins are ferried across the nuclear envelope in a trimeric complex with an importin alpha and an importin beta1 protein. Synthesis of each of the 6 mouse importins is highly regulated throughout spermatogenesis, with mouse importin alpha 2 (KPNA2) transcript levels maximal at E12.5 in the embryonic testis and within spermatocytes and spermatids in adults. To identify cargoes transported by importins at key developmental stages in spermatogenesis, we screened an E12.5 mouse testis cDNA Y2H library and identified Paraspeckle protein 1 (PSPC1) as a KPNA2 cargo. PSPC1 is highly expressed in testes and is the defining marker of paraspeckles, a distinct subnuclear structure formed around a non-coding RNA transcript, NEAT1. Paraspeckles are regarded as a structure within which A-to-I edited RNA transcripts are retained in the nucleus. To address the hypothesis that PSPC1 trafficking into the nucleus and subsequent localisation into paraspeckles is mediated specifically by KPNA2, we made recombinant proteins and used an ELISA-based assay to measure binding between PSPC1 and importin alpha proteins. PSPC1 exhibited remarkable selectivity for binding to KPNA2 and another importin, KPNA6, but not KPNA4. The importance of KPNA2 for PSPC1 nuclear transport was tested by transient transfection of HeLa cells with GFP-tagged full length- and truncated- (dominant negative) importin constructs. Nuclear accumulation of both endogenous PSPC1 (detected by immunostaining) and overexpressed, tagged PSPC1 (following co-transfection of DsRed2-tagged PSPC1) was visualised by scanning laser confocal microscopy and quantified with Imaris software. A significant, direct relationship between the amount of transport-active KPNA2 and paraspeckle number was measured, while paraspeckle size was unchanged. Thus changing importin levels during spermatogenesis may affect cell function by controlling assembly of RNA processing machinery. Antibodies to NONO and PSF revealed the developmentally-regulated localisation of other paraspeckle proteins in the mouse testis. Our findings indicate that paraspeckle composition and number may change during germline development, and that this may be a consequence of regulated importin synthesis. Future studies will test whether paraspeckles contribute to translational regulation during spermatogenesis.

GENETICS

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MEASUREMENT OF 5-METHYLCYTOSINE AND 5-HYDROXYMETHYLCYTOSINE LEVELS OVER TIME IN A DONOR POPULATION AND ABERRATIONS TO GLOBAL DNA METHYLATION QUANTITIES IN AN OLIGOZOOSPERMIC POPULATION

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(Presented by: Timothy Jenkins, BS)

Introduction: Recent data suggest an essential role of the paternal epigenome in spermatogenesis, normal gamete function and embryogenesis. One important epigenetic modification in sperm is DNA methylation, which is associated with gene silencing. Intermediates formed in the demethylation pathway such as 5-hydroxymethylcytosine (5-hmC) are additionally thought to play a regulatory role by allowing regions of DNA, like promoters, which contain 5-hmC to be more quickly activated by full demethylation. The influence of these modifications in the male gamete and their role in infertility has yet to be elucidated.

Objective: Our objective was to quantify 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) levels in sperm DNA from oligozoospermic patients and controls. In addition we observed the effects of aging on 5-mC and 5-hmC levels in sperm donors from 2 different collections times, at least 10 years apart.

Materials and Methods: Sperm DNA isolated from 26 oligozoospermic patients, 26 normozoospermic controls and 15 sperm donors at two different time points were analyzed for global 5-mC and 5-hmC levels. 5-mC and 5-hmC were quantified using a colorimetric ELISA assay (Epigentek Farmingdale, NY). Two- tailed t test and regression analysis were performed using STATA 11 software to analyze significance ($p < 0.05$).

Results: Oligozoospermic patients had a significantly decreased global 5-mC level on average compared to control samples ($p < 0.001$) with 5-mC accounting for approximately 0.76% of the total sperm DNA analyzed while the 5-mC content in normoszoospermic men was 1.55%. 5-hmC levels also appeared to decrease in the oligozoospermic group, however this difference failed to reach significance ($p = 0.227$). Additionally, linear regression analysis revealed a positive correlation with age and 5-mC ($R = 0.475$; $p = 0.008$) as well as 5-hmC ($R = 0.38$; $p = 0.0383$) quantities. However, no correlation was found between 5-hmC and 5-mC levels ($R = 0.168$; $p = 0.374$).
Conclusions: Our data suggest that global sperm DNA demethylation is associated with oligozoospermia in our subset of patients. Specifically, oligozoospermic patients have decreased global 5-mC when compared to normosoospermic men. Additionally, our data suggest that both 5-mC and 5-hmC quantities in sperm may increase with age.
Funding: None.

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ASSESSMENT OF SPERM AND LEUKOCYTE TELOMERE LENGTHS IN ASSOCIATION WITH AGE AND SEMEN PARAMETERS

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(Presented by: Kenneth Aston, PhD)

Standard semen parameters such as sperm concentration, viability and morphology generally decline only moderately with advanced paternal age. Recently, advanced paternal age has been linked to increased risk for neuropsychiatric disorders including autism and schizophrenia. Intriguingly, a protective mechanism for potential negative effects of advanced paternal age on offspring health has been recently proposed. Unlike replicating somatic cells whose telomeres shorten with age, the telomeres of sperm are longer in older men, and offspring from older men display longer leukocyte telomere length (LTL) than do offspring from younger men.

Age-dependent telomere attrition in leukocytes and lengthening in sperm were reported based on cross-sectional studies that examined either LTL or sperm telomere length. However, no study has evaluated age-dependent changes in telomere lengths in leukocytes and sperm from the same individuals. In principle, sperm telomere length of older sperm donors might not represent the lengths in the general male population. Accordingly, we measured telomere lengths in paired samples of sperm and blood from 135 men (age 18-68) by Southern blot analysis of the terminal restriction fragment lengths. Similar to earlier non-paired studies, we observed a positive linear association between age and sperm telomere length ($\beta=0.035\pm0.012$, $p=0.0034$) and a negative linear association between age and LTL ($\beta=-0.019\pm0.005$, $p=0.0004$), with the difference in the two beta coefficients being highly significant at $p<0.0001$ in a repeated measures analysis. Interestingly, while a significant age-adjusted correlation between leukocyte and sperm telomere length within individuals was identified ($r=0.40$, $p<0.0001$), the correlation was weaker than the reported correlations of telomere length among different somatic tissues within an individual, possibly indicating that sperm telomere dynamics are less tightly controlled or more prone to environmental influence than other tissues. Further, in a subsct of 75 subjects with semen analysis data, sperm telomere length was not correlated with standard semen parameters including sperm concentration ($p=0.48$), motility ($p=0.46$), or viability ($p=0.85$). This is the first report of the relationship between sperm telomere length and LTL using paired samples from the same individuals. Future work should evaluate the factors that affect sperm telomere dynamics and their relation to offspring telomere length.

INFERTILITY / ART / MALE CONTRACEPTION

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ROLE OF NON-INVASIVE MARKERS IN PREDICTION OF SPERM RETRIEVAL IN NON- OBSTRUCTIVE AZOOSPERMIA

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(Presented by: Vasan Satya Srini, DNB – Urology)

Objective: To predict the accuracy of sperm retrieval by using such non-invasive markers in order to avoid the morbidities and complications of surgery.
Design: Prospective, non-randomized cohort study.
- Settings: Andrology unit in a Tertiary Fertility Centre, India.
- Patients: 100 consecutive patients diagnosed to have non obstructive azoospermia between January 2009 and December 2010 and undergoing testicular sperm extraction (TESE).
- Interventions: Patients with azoospermia scheduled for TESE: Serum Inhibin B and epididymal head size were measured. The biopsy report after TESE was recorded.

Main Outcome Measure(s): All results thus obtained were tabulated, and correlation of these markers with respect to sperm retrieval were analyzed.
Results: Out of 81 patients in whom serum Inhibin- B values was > 40 pg/ml, 67(82.7%) patients had sperms in TESE. Out of 79 patients in whom epididymal head size was > 6 mm, 64(81.0%) patients had sperms in TESE. Out of 69 Patients in whom epididymal head size was > 6 mm and serum Inhibin- B value was > 40 pg/ml, 62(89.9%) patients had sperms in TESE.
Conclusions: Serum inhibin- B level and epididymal head size are the best non-invasive markers which in combination further increases the predictive accuracy of sperm retrieval with non obstructive azoospermia.
Key Words: Non Obstructive Azoospermia, Testicular Sperm Extraction, Inhibin -B, Epididymal head size, Intra Cytoplasmic Sperm Injection

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THE EFFECT OF THE HYPO-OSMOTIC SWELLING TEST SCORE ON MISCARRIAGE RATES

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(Presented by: Aniela Bollendorf)

Introduction and Objective: Most studies find that low hypo-osmotic swelling (HOS) test scores fail to achieve pregnancies following intercourse, intrauterine insemination (IUI), or conventional oocyte insemination in vitro fertilization-embryo transfer (IVF-ET) unless the sperm pre-treated with chymotrypsin or intracytoplasmic sperm injection (ICSI) is performed with IVF. However one study found pregnancies but a higher miscarriage rate. The purpose of the present study evaluated the effect of low HOS scores following IVF-ET with ICSI on miscarriage rates.

Furthermore it evaluated the effect of grey-zone HOS scores on miscarriage rates with conventional oocyte insemination vs. ICSI.
Methods: Miscarriage rates counted clinical pregnancy, gestational sac without a fetal pole, or fetal demise in the first trimester according to deciles of HOS scores. ICSI was performed in all cycles when HOS scores were <50%. The use of ICSI vs. conventional insemination was dependent on HOS scores. The grey-zone was analyzed according to whether the oocyte was inseminated conventionally vs. ICSI.
Results Obtained: The miscarriage rates including clinical pregnancies (beta-hCG levels >100 mIU/mL with two consecutive rises) according to deciles of HOS scores of 0-19 (first 2 deciles conbined), 29-29, 39-39, and 40-49 were 10.9% (2/22), 11.7% (2/17), 18.3% (11/66) and 27.2% (43/158). For normal HOS deciles of 50-59 (grey-zone), 60-69, 70-79, and 80-89 the miscarriage rates were 22.9% (70/310), 28.0% (172/616), 27.0% (270/1000) and 24.9% (118/493). Evaluating the grey zone according to conventional oocyte insemination vs. ICSI the miscarriage rates were 19.7% (19/96) vs. 22.4% (48/214).
Conclusions: ICSI not only allows the achievement of pregnancies with sperm with low HOS test scores <50% but does not lead to a higher miscarriage rate. The grey zone of 50-59% was not found in the past to be associated with lower pregnancy rates with intercourse, IUI or IVF with conventional oocyte insemination. These data show no increased risk of miscarriage with conventional oocyte insemination either.

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THE CORRELATION OF THE DEGREE OF ABNORMAL SPERM MORPHOLOGY USING STRICT CRITERIA AND PREGNANCY RATES FOLLOWING INTRAUTERINE INSEMINATION (IUI)

Jerome Check, Mary Yurashevich and Aniela Bollendorf
(Presented by: Jerome Check)

Introduction and Objective: Despite initial enthusiasm that males with sperm with <4% normal morphology using strict criteria are markedly subfertile some subsequent studies failed to corroborate this as a discriminatory level to identify the subfertile male. The possibility exists that lower levels of morphology can identify the subfertile male but the majority of males have levels toward the upper side of this 4% level and thus fails to identify the subfertile male. The purpose of this study was to determine if very low percentage of normal morphology does correlate with subfertility.
Methods: A retrospective review of women aged <35 undergoing IUI over a 2 year period was performed. The clinical and ongoing delivered pregnancy rates (PRs) per IUI cycle were determined at 6 different levels of morphology – 0, 1, 2, 3, 4, 5, and >6%. Motile densities were all normal.
Results Obtained: The clinical (ultrasound evidence of pregnancy at 8 weeks) was as follows: 0% – 2/21 (9.5%), 1% – 7/30 (23.3%), 2% – 4/34 (11.8%), 3% (5/31 (16.1%), 4% – 9/70 (12.9%), 5% – 8/65 (12.3%), and >6% – 54/403 (13.4%). The ongoing/live delivered pregnancy rates were 9.5%, 16.7%, 8.8%, 16.1%, 11.4%, 12.3%, and 10.9%.
Conclusions: The failure of low strict morphology to detect a subfertile male was confirmed by this study. Furthermore there does not appear to be any low level that is definitely associated with subfertility. Further studies will look at specific morphologic abnormalities to see if a particular defect can better identify the subfertile male.

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EFFECT OF AGE ON PREGNANCY RATES PER CYCLE OF INTRAUTERINE INSEMINATION FOR MALE FACTOR IN NATURAL CYCLES SUPPORTED BY LUTEAL PHASE SUPPLEMENTATION

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(Presented by: Jerome Check)

Introduction and Objective: Intrauterine insemination (IUI) of sperm may improve the chance for conception in couples where a male factor was considered as the main cause of infertility other than female age, by allowing concentrated sperm to enter the uterine cavity shortly after oocyte release which would not be possible following intercourse since the mucus quality would likely have waned. The present study evaluated the effect of female partner's age on IUI outcome.
Methods: Couples with a male factor problem either related to motile density <10x106/mL, <30% motility irrespective of concentration, or antisperm antibodies (ASA) >80%, or hypo-osmotic swelling (HOS) test <50% were treated with IUI with documented oocyte release by ultrasound. A requirement was attaining a follicle of >18mm diameter with a serum estradiol >200 pg/mL in a natural cycle. For ASA or low HOS the sperm was pretreated first with chymotrypsin
Results Obtained:
Pregnancy rate for natural cycle IUI's

Age at IUI of female partner	<35	36–39	40	41	42	43	44	>45
# IUI's	376	256	70	44	31	24	24	32
# clinical preg.	31	21	2	3	3	2	1	0
% clinical preg./transfer	8.2	8.2	2.9	6.8	9.7	8.3	4.2	0.0
% viable at end of 1st trimester	28	15	2	2	3	3	0	0
% viable at end of 1st trimester/ transfer	7.4	5.9	2.9	4.5	9.7	12.5	0.0	0.0
% miscarriage/ clin. preg.	12.9	33.3	50.0	33.3	33.3	50.0	100.0	
% delivered/ ongoing	7.4	5.1	1.4	4.542	6.5	4.2	0.0	0.0

Conclusions: There were 6 live deliveries in 169 IUI cycles in women 40-44 (3.6%). Thus the live delivered pregnancy rates per cycle was half as good as young women <35. Since these women did not take expensive follicle maturing drugs (just inexpensive progesterone), IUI for male factor in the older age group is a reasonable option and can lend itself to multiple inexpensive cycles. Since some studies show the highest pregnancy rate in the first or second IUI cycle this study purposely evaluated all IVF cycles. Some even had 10 or more IUI's. Though IVF-ET is likely to result in a higher pregnancy rate, the advanced age group notoriously has low pregnancy rates per transfer relative to younger ages at a much higher expense. Even if it would take multiple IUI cycles to achieve a pregnancy it is likely to be far less expense then even one IVF-ET cycle.

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EXPERIENCE WITH PERCUTANEOUS SPERM ASPIRATION (PESA) AND INTRACYTOPLASMIC SPERM INJECTION (ICSI) FOR MAN WITH VASECTOMY: ANALYSIS OF PROGNOSTIC FACTORS FOR PREGNANCY AND LIVE BIRTH

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(Presented by: Marcelo Vieira, MD; Urologist)

Introduction: Sperm retrieval by PESA and ICSI has become a routine option for treatment of obstructive azoospermia. Vasectomy and failed vasectomy reversal are the most common reasons for the use of this technique. The literature shows a list of prognostic factors for pregnancy and live birth that includes female age, interval after vasectomy, number of oocyte manipulated in the laboratory, fertilization rate and number of good embryos. Interval after vasectomy is one of the prognostic factors of influence and is inversely related to pregnancy rate and live birth. The objective of this study is evaluate those parameters as prognostic factors for pregnancy and live birth.

Methods: We retrospectively studied the charts of 333 PESA and ICSI cycles for treatment of obstructive azoospermia due to vasectomy or failed vasectomy reversal from three different Brazilian assisted reproduction centers covering a eleven years period. Our data bank included the variables: female and male age; interval after vasectomy; number of oocyte manipulated by the laboratory; fertilization rate; number of good embryos; pregnancy rate and live birth. For the study of interval after vasectomy as a prognostic factor we divided patients in four groups: GI– less than 3 years (n=02); GII–3 to 8 years (n=51); GIII– 9 to 14 years (n=124); GIV–15 or more years (n=156). We used 19.0 version of SPSS (Statistical Package for Social Sciences) for statistical analysis adopting 5% for significant level (p<0,05).

Results: The median interval after vasectomy for non pregnancy couples was 14,29±6,00 years and for pregnancy couples was 14,87±6,13 years (p=0,427). For live birth the median values were respectively 14,04±6,00 and 15,16±6,12 (p=0,219). Group I was excluded of the analysis because of the smal number of patients. Comparison of pregnacy and live birth among groups II, III and IV showed no statistical significance.

Female age, number of fertlized oocyte and number of good embryos reached statistical significance as a prognostic factors for pregnancy and live birth.

Conclusions: Interval time after vasectomy did not correlate significantly with pregnancy and live birth.

Female partner and laboratory variables were prognostic factors for pregnancy and live birth for treatment of obstructive azoospermia due to vasectomy and failed vasectomy reversal.

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ROLE OF SPERM FACTORS IN IDIOPATHIC RECURRENT SPONTANEOUS ABORTION

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AIIMS

(Presented by: Kishlay Kumar, MSc)

Introduction: Recurrent spontaneous abortion (RSA), defined as the occurrence of three or more consecutive pregnancy failures, is estimated to occur in 2–3% of all couples. 50% of these cases are idiopathic (iRSA). This study was aimed to examine the protamine 2 (PRM2) transcript level and sperm DNA damage in iRSA cases.

Objective: The study was designed to examine the relationship between sperm chromatin integrity and PRM2 transcripts in couples with iRSA. Thirty couples and 20 fertile controls were included in the study. Semen samples were collected by masturbation from 30 men (mean age of 35±5 years) after 4 days of abstinence. Semen analysis was performed as per WHO (2010) guidelines.

Materials and Methods: DNA fragmentation was analysed by SCSA. SCSA results were expressed as DNA fractionation index (DFI) and highly DNA stainable (HDS) cell fractions. The X–mean, Y–mean values were recorded manually and percentage of DFI was calculated. For expression study, sperm were separated using separation media and RNA was extracted using RNAeasy mini kit. cDNA was prepared and amplified using specific primers for each gene to verify sperm cDNA presence. Then PRM2 expression was quantified by real–time PCR using SYBR Green PCR Master Mix.

Results: iRSA and control groups did not show any significant differences in the sperm parameters. However, using receiver operating curve (ROC) analysis a cut off value of approximately 26% DFI was found in cases with iRSA. The level of PRM2 transcript was estimated using relative quantification and was found to be significantly ($p<0.05$) less expressed in iRSA cases.

Conclusion: Low level of PRM2 may be associated with increased risk of pregnancy loss. It is possible that low PRM2 transcript level may lead to defective chromatin packaging and subsequently increased DNA damage. Also it may be hypothesized that PRM2 deficiency could be a cause for sperm DNA damage and embryo death in cases of iRSA.

Funding: Financial support from Department of Biotechnology (DBT), India is highly acknowledged.

MALE SEXUAL FUNCTION

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THE NONSTEROIDAL, ANTIESTROGEN TAMOXIFEN, BLOCKS 17-BETA-ESTRADIOL INDUCED RELAXING EFFECTS THROUGH NON-GENOMIC PATHWAYS IN HUMAN AND RAT CORPUS CAVERNOSUM

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(Presented by: Ege Can Serefoglu, MD)

Introduction and Objectives: Declining levels of testosterone (T) and sexual quality-of-life is a concern for aging men. Concomitant with T reduction, these aging men often have higher estrogen (E2) levels, 75% to 90% of which derives from peripheral aromatization. This current research examined the non-genomic relaxant effect of this "female" steroid hormone, 17-beta-estradiol (17β-E2), on human and rat corpus cavernosum (CC) smooth muscle.
Methods: Human CC specimens were obtained from patients undergoing penile prosthesis surgery (age range 58-61, n=12) after local IRB approval. Isolated strips from cavernosal tissues of human and adult male Sprague-Dawley rats (n=9) were suspended in organ baths for isometric recordings. CC relaxation was expressed as the percent decrease in precontraction induced by phenylephrine (PE, 10-5 M). After precontraction of PE, the strips were treated with increasing concentrations (10-8 to 10-4 M) of 17β-E2. In addition, the relaxation studies were repeated using tamoxifen (estrogen receptor antagonist, 10-5 M), N(G)-nitro-L-arginine methyl ester (L-NAME, NO synthesis inhibitor, 10-4 M), 1H-[1,2,4] oxadiazolol [4,3,a] quinoxalin-1-one (ODQ; a guanilyl cyclase inhibitor 10-6 M), glibenclamide (K+(ATP) channel inhibitor, 10-5 M) or 8-Bromo-cGMP (cell permeable cGMP analog, 10-6 M) for 15â€‰min.
Results: 17β-E2 demonstrated a dose dependent decrease in tension induced by 10 μM PE. The maximum response (80.8 ± 8.21%, at 10-4 M) to 17β-E2 revealed 97% decrease by tamoxifen and a 93% decline by glibenclamide in HCC. In rat CC, 17β-E2 induced maximum relaxation of 77 ± 7.94% which were inhibited 54% by tamoxifen and 67 % by glibenclamide. L- NAME, ODQ and 8-Bromo-cGMP did not affect 17β-E2 induced relaxation in both CC tissues.
Conclusions: This data set provides the first description of a non-genomic relaxing effect of 17β-E2 on both human and rat cavernosal tissue. 17β-E2 reduced CC tone, probably through receptor-mediated relaxation involving K+ (ATP) channels and reduction of calcium entry, but not the NO-NOS-cGMP pathway. The tamoxifen mediated antagonism of the relaxation response by 17β-E2 was greater in human than in rat CC. Long-term estrogen exposure as witnessed in adult onset hypogonadism may directly affect cavernosal tissues leading to decreased human erectile tissue activity, a deterioration on certain components of sexual behavior and progression of ED.

OTHER

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IDENTIFICATION OF A VOLTAGE ACTIVATED K+ CHANNEL IN HUMAN SPERMATOZOA BY WHOLE CELL PATCH CLAMPING

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(Presented by: Steven Mansell, BSc)

Introduction: Ion channels are important in normal sperm physiology since they regulate membrane potential, intracellular pH and Ca2+ entry. Recently with the successful application of sperm whole cell patch clamping we are starting to explore the characteristics of these channels, and their role in male fertility. The aim of this study was to use whole cell patch clamping to elucidate and characterise ion channels in human spermatozoa under quasi-physiological conditions.
Methods: Semen samples were obtained from a cohort of healthy donors with no obvious fertility issues (Ethical approval number 09/s1402/6) and normal semen parameters (WHO 2010). Sperm isolated by swim up were incubated under capacitating conditions for 4 hours (25mM NaHCO3, 6% CO2 at 37°C). Currents recorded by ramp changes in holding potential (-80 to 80mV, Vhold =-80mV) were analysed from cells under voltage clamp in whole cell configuration over a 250ms period. Standard pipette and bath solutions were designed to mimic quasi-physiological ionic conditions ([Na+]o = 135mM [K+]i =113 mM, [K+]o = 4.7 mM pH 7.4.
Results: The membrane potential (Vm) under resting conditions was -40.0 ± 4.0mV (n=10). Data also showed an outwardly rectifying current only active when depolarised past resting Vm (>-40mV). Raising [K+]o to 130mM depolarised Vm to -2.5 ± 5mV toward EK (n=9, p= 0.001). Moreover, replacing pipette K+ with Cs+ (n=8) consistently abolished the depolarization-induced current and shifted Vm to 2.9 ± 5mV. Cytoplasmic acidification from [pH]i 7.4 to [pH]i 6.8 caused a decrease in the depolarised outward current (~50%) suggesting an important link between [pH]i and channel activation. Channel inhibition by intracellular acidification had no effect on resting Vm.
Conclusion: This is the first time resting membrane potential has been assessed using electrophysiology in sperm cells under quasi-physiological conditions. We have identified a voltage activated K+ channel in spermatozoa that is inactive at resting Vm, indicating other conductances are involved in regulating Vm, this was further confirmed by intracellular acidification. However, the role of these channels in male fertility remains to be explored.
Funding: Wellcome Trust, NHS Tayside and Infertility Research Trust

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PROTEIN PROFILING OF SEMEN SAMPLES IN PATIENTS UNDERGOING FERTILITY EVALUATION

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Create Fertility Centre
(Presented by: Shlomit Kenigsberg, PhD)

Introduction: The laboratory evaluation of male infertility remains an essential area of research as almost one third of cases are classified as idiopathic. The clinical value of traditional semen parameters in the diagnosis of male infertility is controversial as normoozospermia does not guaranty successful fertilization. A proportion of male infertility could be caused by

molecular factors including the absence of expression or atypical localization of some proteins. This highlights the importance of identification of sperm proteins affiliated to fertilization. The objective of this study was to examine the relationship between proteins content to sperm concentration, motility and DNA damage in patient undergoing fertility evaluation.
Materials and Methods: Following Institutional Research Ethics Board approval, semen samples from 41 patients undergoing infertility evaluation were assessed by computer-assisted semen analysis (CASA). Sperm DNA damage was evaluated by flow cytometry based technique with acridine orange and expressed as DMA Fragmentation Index (DFI). Sperm proteins were extracted by two methods using NP40 for separation of non-soluble and acrosomal-soluble extracts and modified RIPA buffer. Samples were separated using SDS-electrophoresis and visualized by staining with Gelcode and scanning using the Li-cor Odessey scanner.
Results: In this preliminary study, clear differences in sperm proteins with molecular weight (< 17KDa) were observed in patients with abnormalities in standard semen parameters in comparison to normozoospermic patients with. The correlation between levels of sperm DNA damage and proteins profile were also noticeable.
Conclusion: Novel non-ionic detergent NP40 is a suitable method of protein extraction from human ejaculates. Semen quality and levels of DNA damage are associated with differences in protein content. Further research is needed in order to characterize these proteins and to determine whether these proteins are nuclear or DNA binding proteins.

PROSTATE / TESTIS CANCER / CLINICAL UROLOGY

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DOES AGE AFFECT THE EFFICACY AND SAFETY OF GREENLIGHT HPS™ LASER PHOTOSELECTIVE VAPORIZATION PROSTATECTOMY (PVP)?

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(Presented by: Carson Wong, MD)

Background: To evaluate the efficacy and safety of GreenLight HPSä laser photoselective vaporization prostatectomy (PVP) for the treatment of benign prostatic hyperplasia (BPH) in patients of different age groups.
Methods: 164 consecutive patients were stratified into two groups: age<70 (group I, n=93) and age≥70 (group II, n=71) years. Transurethral PVP was performed using a GreenLight HPS™ side-firing laser system. Voiding parameters were measured preoperatively and at 1 and 4 weeks and 3, 6, 12, 18, 24 and 36 months postoperatively.
Results: Among the preoperative parameters evaluated, there were significant differences ($p<0.05$) in prostate volume (I: 58.7; II: 73.6 mL) and serum PSA (I: 1.9; II: 2.9 ng/mL), while American Urological Association Symptom Score (AUASS), Quality of Life (QoL), maximum flow rate (Qmax), Sexual Health Inventory for Men (SHIM) and post void residual (PVR) were similar ($p>0.05$) between groups. No significant differences in laser utilization, energy usage and operating time were noted. Clinical outcomes (AUASS, QoL, Qmax, PVR) showed immediate and stable improvement from baseline ($p<0.05$) within each group, but no significant differences between the two groups were observed during the follow-up period. The incidence of adverse events was low and similar in both groups.
Conclusions: The results suggest that age has little effect on the efficacy and safety of GreenLight HPS™ laser PVP.

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DOES PROSTATE CONFIGURATION AFFECT THE EFFICACY AND SAFETY OF GREENLIGHT HPS™ LASER PHOTOSELECTIVE VAPORIZATION PROSTATECTOMY (PVP)?

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(Presented by: Carson Wong, MD)

Objective: We evaluate the efficacy and safety of GreenLight HPSä laser photoselective vaporization prostatectomy (PVP) for the treatment of benign prostatic hyperplasia (BPH) with different prostate configuration.
Materials and Methods: Patients were stratified into two groups: bilobe (group I) and trilobe (group II) BPH. Transurethral PVP was performed using a 120W GreenLight HPS™ side-firing laser system. American Urological Association Symptom Score (AUASS), Quality of Life (QoL) score, maximum flow rate (Qmax) and post void residual (PVR) were measured preoperatively and at 1 and 4 weeks and 3, 6, 12, 18, 24 and 36 months postoperatively.
Results: 160 consecutive patients were identified (I: 86, II: 74). Among the preoperative parameters, there were significant differences ($p<0.05$) in prostate volume (I: 46.0 ± 19.8; II: 87.5 ± 39.6 mL), Qmax (I: 9.9 ± 3.9; II: 8.7 ± 3.5 mL/sec), PVR (I: 59.2 ± 124.6; II: 97.7 ± 119.1 mL) and PSA (I: 1.4 ± 1.4; II: 3.6 ± 2.6 ng/mL), while AUASS and QoL were similar ($p>0.05$). Significant differences ($p<0.05$) in laser utilization (I: 9.5 ± 6.6; II: 19.5 ± 11.6 minutes) and energy usage (I: 63.1 ± 43.9; II: 132.5 ± 81.1 kJ) were noted. Clinical outcomes (AUASS, QoL, Qmax and PVR) showed immediate and stable improvement from baseline ($p<0.05$) within each group, but no significant differences between the two groups were observed during the follow-up period ($p>0.05$). The incidences of adverse events were low and similar in both groups.
Conclusions: Our experience suggests that BPH configuration has little effect on the efficacy and safety of GreenLight HPS™ laser PVP.

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TISSUE PROTEOMICS TO DISCOVER BIOMARKERS OF TESTICULAR TUMOURS

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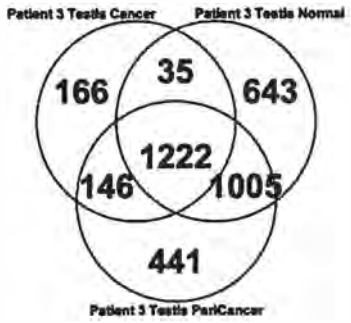
(Presented by: Mohammed Mosli MBBS)

Introduction and Objective: To date there are no bio-markers that are specific for sub-types of testis cancer (Gilligan et al, J Clin Oncol. July, 2010). Our objective is to discover new bio-markers for testicular cancer which would allow us to identify cancer type prior to orchidectomy. We hypothesize that testis cancer proteomics would allow us to identify biomarkers for testis cancer. Li et al has previously reported the detection of 725 unique testis proteins from normal testis. We have optimized the tissue protein extraction and Mass Spectrometry (MS) analysis techniques and now are able to detect over 2900 proteins in normal testicular tissue. Our specific objective for this study was to identify the proteome of testis cancer, peri-cancerous tissue and normal testis tissue.
Methods: Fresh tissue was immediately placed in phosphate buffer saline with protease inhibitor cocktail, serum components were depleted with the use of a haemolysis buffer, proteins were extracted for MS analysis using a Surfactant reagent (RapiGest™ SF) then deactivated by acidification to a pH=3. The samples were then fractionated using a Strong Cation Exchange (SCX) column then loaded into High Pressure Liquid Chromatography coupled online to a LTQ-Orbitrap MS. Bioinformatic analysis was performed using Mascot, Xtandem and Scaffold software.
Results: We have identified 2905 proteins from near normal tissue, 2814 proteins from peri-cancerous tissue and 1569 protein from cancerous tissue of which 166 were unique to Cancer, 146 were unique to cancerous and peri-cancerous tissue and 643 were unique to normal tissue.
Conclusions: This is the first report that we are aware of on testicular cancer proteomics. The proteome of the testis cancer is distinctly different than the proteome of normal testis tissue and leaves us with literally 100's of potential bio-markers for testicular cancer.



SPERM FUNCTION / SEMEN ANALYSIS

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PRODUCTION AND CHARACTERIZATION OF RECOMBINANT HUMAN BINDER OF SPERM PROTEIN HOMOLOG 1 (BSPH1)

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(Presented by: Niaz Oliazadeh, PhD)

Our laboratory has demonstrated the importance of Binder of SPerm (BSP) proteins in bovine and porcine sperm capacitation. Recent studies identified a BSP-homologous DNA sequence in the human genome (BSPH1). We hypothesize that the human BSP homolog is added to sperm during epididymal maturation and play an analogous role in sperm functions in this species. Due to low concentration in seminal plasma, native protein is not available. The first objective was soluble expression of recombinant BSPH1 (recBSPH1) to allow downstream studies. His6-thioredoxin (TRX)-tagged protein was expressed in Origami B-(DE3) using pET32-a vector. On-column refolding and purification procedure was optimized with immobilized metal ion affinity chromatography. The tag part (His6-TRX) was cleaved using Enterokinase and the cleaved protein was purified with His-Tag column. The purity of rBSPH1 protein was about 95% and the purification method was reproducible (3 mg/liter culture). The identity of the tag-free recBSPH1 was confirmed by amino-terminal sequencing and mass spectrometry. BSP family proteins have some specific, well determined binding characteristics, such as binding to heparin, gelatin, low and high density lipoprotein, phosphatidylcholine and human sperm. Most of the recBSPH1 (90%) interacted with heparin-Sepharose, and could be eluted with Tris-buffer containing 1 M NaCl. About 30% of recBSPH1 bound to gelatine-agarose column strongly and could be eluted with 8 M urea. The availability of tag-free highly purified recBSPH1 should help us to establish its role in human sperm functions and fertility.
Funding: Supported by RQR, FESP University of Montréal, CIHR.

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IDENTIFICATION OF SUMOYLATED PROTEINS IN HUMAN SPERM

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(Presented by: Margarita Vigodner, PhD)

Introduction: Our group continues to characterize the role of Small Ubiquitin-like Modifiers or SUMO proteins and sumoylation (covalent modification by SUMO) in male germ cells and sperm. In human sperm, sumoylated proteins are enriched in the neck area of the cells and can be detected at low level in other parts of the cells, but their functions in sperm are unknown.
Methods: SDS extraction of human sperm proteins was followed by immunoprecipitation with anti-SUMO antibodies and mass spectrometry analysis to identify sumoylated proteins.
Results: About fifty sperm proteins were identified as SUMO-targets. Among the identified proteins were several glycolytic and mitochondrial enzymes, structural components of the fibrous sheet and outer dense fibers, actin-binding proteins, molecular chaperones including heat shock proteins, and several other proteins implicated in capacitation and acrosome reaction in human sperm. Some proteins previously localized to the redundant nuclear envelope were found sumoylated, a finding which is consistent with the enrichment of sumoylated proteins in this sperm area. Interestingly, many of the identified proteins were previously reported to be phosphorylated during sperm capacitation and /or modified by nitosylation. Induction of sperm capacitation caused significant changes in the overall level of sumoylation proteins.
Conclusion: Taken together our data suggest that sumoylation together with other posttranslational modifications play important roles in the regulation of sperm functions.

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SEMINAL REACTIVE OXYGEN SPECIES ESTIMATION: IS SINGLE READING ENOUGH? CLINICAL IMPLICATIONS

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(Presented by: Rima Dada MD, PhD)

Introduction: The finding that 10–15% infertile men have normal spermiogram, have emphasized the need to develop biomarkers for accurate prediction of sperm reproductive capacity. Reactive oxygen species (ROS) and sperm DNA damage are reported as promising tool in diagnostic evaluation, with studies implicating them in reduced fertility, impaired embryo development and higher rates of miscarriages. Recently revised WHO 2010 guidelines have also recognized the importance of ROS and sperm DNA damage and many andrology laboratories across the world have started quantifying ROS and sperm DNA damage as a supplement to semen analysis. This is the first study reporting intra individual variations in ROS levels, which could lead to improper assessment of oxidative stress (OS) induced damage and have serious clinical implications.
Material and Method: Semen was collected twice after an interval of 2 weeks from 35 idiopathic infertile men and 27 fertile controls. ROS in neat semen was quantified by luminol induced chemiluminescence. DNA fragmentation index (DFI) was assessed by sperm chromatin structure assay (SCSA). Statistical difference was calculated by Mann–Whitney test using SPSS 11.5 software and P–value <0.05 was considered significant.
Results: Mean ROS levels in infertile men was 14213.12 RLU/min/20 million sperm and 19278.35 RLU/min/20 million sperm in first and second semen sample respectively. ROS levels in controls were 1426.23 and 1095.29 RLU/min/20 million sperm in first and second semen sample respectively. The intra–individual ROS variation between both semen samples was statistically significant ($p<0.05$). However there was insignificant difference in DFI between the first and second sample of cases and controls ($p>0.05$).
Discussion: Recent publications have highlighted importance of sperm DNA damage and ROS assessment to elucidate the etiology and quantum of OS and in optimizing the dose of antioxidants administered to patient. Our findings are significant because intra–individual variation in ROS levels lead to wrong estimation of magnitude of OS, due to which inappropriate antioxidant dose may be advised. As a result ROS levels may reduce below physiological threshold and normal sperm functioning may be impaired. In consideration of significant difference of ROS levels between two semen ejaculates, it is important that mean of 2 readings which are analyzed at least at 1 month interval should be considered in diagnostic workup of infertile men.

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ASSESSMENT OF HUMAN SPERM CHROMATIN INTEGRITY AND DISTRIBUTION OF NUCLEAR SULFHYDRYL GROUPS BEFORE AND AFTER VARICOCELE REPAIR: A PROSPECTIVE TRIAL

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(Presented by: Naif Alhathal, MD)

Introduction: Human sperm DNA damage may adversely affect reproductive outcomes, and the spermatozoa of infertile men possess substantially more DNA damage than that of fertile men. Further to our previous study on the effect of varicocelectomy on sperm DNA integrity using the sperm chromatin structure assay (SCSA), the objective of this study was to assess the distribution of sperm nuclear sulfhydryl groups before and after varicocelectomy.
Materials and Methods: We evaluated 21 men who underwent microsurgical varicocelectomy for clinical varicocele and 5 controls (sperm donors) at our institution. We examined standard semen parameters, sperm chromatin and DNA integrity (measured by SCSA and expressed as % high DNA stainability – HDS and %DNA fragmentation index – DFI), and, nuclear free sulfhydryl groups (by microscopic evaluation of 5–iodoacetamidofluoresceine, 5–IAF staining) before and after varicocelectomy.
Results: The percentage of spermatozoa with positive 5–IAF staining (diffuse and intense head staining), the %HDS (a measure of chromatin compaction) and the sperm %DFI were all significantly lower in the control group compared to men with varicocele (1.7 vs. 16.3%, 4.0 vs. 9.6%, and 8 vs. 20%, respectively). The percentage of spermatozoa with positive 5–IAF nuclear staining decreased significantly after surgery (from 16.3 to 5.4%). Similarly, the %HDS and %DFI also decreased significantly after surgery (from 10 to 6% and from 20 to 13%, respectively). There were no significant relationships between 5–IAF staining, %DFI and %HDS. However, both IAF staining and %DFI were inversely correlated with motility ($r=-0.44$ and -0.43, respectively).
Conclusion: The data show that varicocelectomy is associated with a significant improvement in sperm DNA and chromatin integrity and a significant reduction in free nuclear sulfhydryl groups. The enhanced chromatin compaction and sulfhydryl group oxidation that is observed after varicocelectomy likely contributes to reducing sperm DNA damage.

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DIFFERENTIAL CYTOKINE EXPRESSION IN LEUKOSPERMIC AND NON-LEUKOSPERMIC SUBJECTS AS A NOVEL TOOL TO PREDICT TREATMENT RESPONSE

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(Presented by: Sruti Chandra, PhD)

Introduction: The infiltration of white blood cells (WBC) especially the polymorphonuclear neutrophils (PMN) in semen (i.e., leukocytospermia) as a result of inflammation of male accessory glands leads to increased oxidative stress with associated decrease in sperm number, motility, and increase in sperm DNA damage. This has been implicated in male factor infertility. Such seminal inflammatory response results in increased levels of IL–4, IL–6, TNF–α and many other chemokines that in turn regulate cytokine expression via positive feedback. Acute or chronic antibiotics treatment currently recommended may help only the responders but there are

many non-responders to such therapy. The present study evaluates selective cytokines expression profile in an attempt to understand their potential mechanistic role during leukocytospermia.

Materials and Methods: Semen samples were collected from age-matched non-leukospermic and leukospermic infertile patients with their consent. These were evaluated for sperm count, motility, progression, morphology, and number of leukocytes as per WHO guidelines. Differential expression profile of 60 cytokines using 'Array6' was determined in the aforesaid samples using RayBio Human Cytokine Antibody Array C series 1000 protein profiling system following kit instructions. Comparative quantitative expression profile was performed by enhanced chemiluminescence and densitometric analysis.

Results: The data analysis of 'Array6' showed upregulation of Angiogenin, BDNF, EGF and IGFBP-4 to various degrees in leukospermic patients who did not respond to antibiotics treatment when compared to those with non-leukocytospermia. Surprisingly, no such upregulation was observed in expression of many known inflammatory chemokines (like IL-2, IL-8, TNF-α etc.).

Conclusions: Such differential cytokine expression profiling, when applied to larger population, differentiates various subsets of responders and non-responders. This may help improve etiology and provide better treatment options in managing male infertility issues of such difficult and frustrated non-responders.

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SPERM KINETICS FOLLOWING VASECTOMY REVERSAL

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(Presented by: Amy Perkins, MS)

Microsurgical vasectomy reversal (VR) techniques are highly successful in restoring sperm to the ejaculate of vasectomized men. Microsurgical vasovasoctomy has been reported to return sperm to the ejaculate in up to 97% of men. The standard length of time a patient can expect for their sperm counts to return to at least 10 million motile has not been previously addressed. The purpose of this study was to conduct a retrospective analysis of records from 33 subjects that underwent a bilateral vasovasoctomy at a specialized vasectomy reversal clinic and then submitted monthly ejaculates for analysis. The average years from vasectomy to reversal for the study group was 8 years (range 1-20) and average age at the time of VR was 40 years (range 30-57). Data included days from VR to analysis, total count, total motile, motility, forward progression and agglutination. All patients produced motile sperm in at least one semen analysis (SA) by the third test, 112 days, with 88% (29/33) ejaculating over 10 million motile sperm. The highest total count and total motile were reported on average within the sixth month (182 days) post operatively with 117 million and 61 million respectively. The relationship between sperm kinetics and subsequent analyses were evaluated with no significant differences noted in total count, total motile, motility, forward progression or agglutination. Sperm kinetics from the first month were compared to the highest report SA values showing an increase ($p<0.05$) in total count, total motile, motility and forward progression. Motility on average returned to 54% with rapid forward progression and insignificant agglutination. Within the six month observation period 42% (14/33) of the subjects reported a pregnancy. Patients that undergo bilateral vasovasostomy can expect sperm return on the first analysis one month post-operatively with a gradual increase in sperm kinetics and a 42% pregnancy rate in the first six months.

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CSRC IS NECESSARY FOR EPIDIDYMAL DEVELOPMENT AND IS INCORPORATED INTO SPERM DURING EPIDIDYMAL TRANSIT

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University of Massachusetts
(Presented by: Pablo Visconti, PhD)

Changes that occur to mammalian sperm upon epididymal transit and maturation render these cells capable of moving progressively and capacitating. Signaling events leading to mammalian sperm capacitation depend on the modulation of proteins by phosphorylation and dephosphorylation cascades. Recent experiments have demonstrated that the Src family of kinases plays an important role in the regulation of these events. However, sperm from cSrc null mice display normal tyrosine phosphorylation associated with capacitation. We report here that, despite normal phosphorylation, sperm from cSrc null mice display a severe reduction in forward motility, and are unable to fertilize in vitro. Histological analysis of seminiferous tubules in the testes, caput and corpus epididymis do not reveal obvious defects. However, the cauda epididymis is significantly smaller, and expression of key transport proteins in the epithelial cells lining this region is reduced in cSrc null mice compared to wild type littermates. Although previously, we and others have shown the presence of cSrc in sperm, a closer evaluation indicates that this tyrosine kinase is not present in sperm from the caput epididymis, suggesting that this protein is acquired by sperm later during epididymal maturation. Consistent with this observation, cSrc is enriched in vesicles released by the epididymal epithelium known as epididymosomes. Altogether, these observations indicate that cSrc is essential for cauda epididymal development and strongly suggest an essential role of this kinase in epididymal sperm maturation.

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SEMINAL QUALITY DECREASES OVER THE 9 YEARS IN SAO PAULO CITY.

Rosa Alice Monteiro, BSc, Juliana Pariz, MSc, Patrícia Pieri, PhD, Paulo Saldiva, PhD and Jorge Hallak, PhD
(Presented by: Jorge Hallak, PhD)

Introduction: Infertility is present in 15% of sexually active couples, affecting about 7% of the entire adult male population. During the last decades, studies have observed a significant decrease of 50% in sperm concentration, showing that semen quality declined, contributing to the recent decrease in fertility rates.

Objective: To determinate changes in semen quality in a group of fertile men, who have undergone vasectomy at the Urology Clinic in the past 9 years.

Methods: In this retrospective study, were included seminal analysis of 743 fertile patients aged between 23 and 50, participants of the voluntary sterilization program at the Urology Clinic of Hospital das Clinicas, University of Sao Paulo (FMUSP) between the years 2000 and 2008. Data were collected from information available in the medical file. Sperm concentration, motility and morphology were assessed following the World Health Organization protocols. Descriptive statistics were performed to observe the measures of central tendency (mean and mode). The correlations were established for these parameters using Pearson's correlation coefficient and was determined $p<0.05$.

Results: Sperm concentration (r= 0,006, p= 0,079), progressive motile (r= −0,18, p< 0,001) and morphology (WHO r=−0,01, p=0,749) presented normal distribution in relation to age of patients. There were significant reduction of progressive motility (r=−0.45 p<0.0001) over the years and morphologically the samples were bellow the criteria of normality, indicating reduced sperm quality. No changes were observed in sperm concentration.

Conclusion: Corroborating recent meta−analysis in others countries, the sperm quality of men in Sao Paulo seems to be decreasing, especially with regard to sperm motility, suggesting that in the future, this scenario may contribute to a marked decrease in male fertility.

Key words: Seminal quality. Sperm. Concentration. Progressive motility. Morphology.

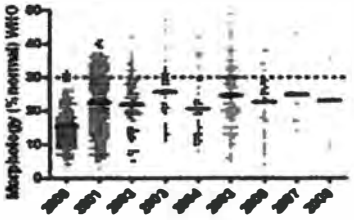

Figure 1. Morphological analysis (red line) were bellow the criteria of normality is according World Health Organization (blue line) over the years.

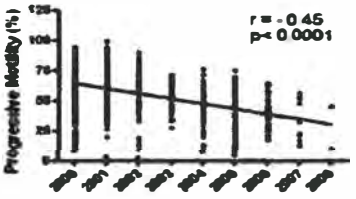

Figure 2. Negative correlation between sperm motility of patients in the years analyzed.

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SPERM ION CHANNELS AS TARGETS FOR MALE CONTRACEPTION

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(Presented by: James F. Smith, MD, MS)

Abstract: Sperm intracellular pH and calcium are two key factors that control sperm activity and fertilizing ability. Elevation of sperm intracellular pH is accompanied by the rise in flagellar calcium, and these events trigger sperm motility hyperactivation and chemotaxis that are critical for sperm ability to find and fertilize the egg. Sperm intracellular Ca^{2+} and pH are controlled by ion channels and transporters such as Hv1, CatSper and sNHE. Interestingly, sperm ion channels and transporters are regulated by certain cues of the male and female reproductive tracts. Physiological regulators such as anandamide, progesterone, prostaglandins as well as toxic compounds such as bisphenols modulate sperm channel activity and determine the fate of fertilization. We will present an up-to-date overview of ion channel-based signal transduction pathways of human sperm cells and discuss new data about physiological regulation of these ion channels. In particular we will discuss regulation of CatSper and Hv1 channel by different components of seminal plasma and steroid hormones found in the female reproductive tract. Targeting ion channels and transporters in order to decrease or abolish sperm motility represents an attractive approach to develop new class of contraceptives

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BULL SPERM PLASMA MEMBRANE PROTEOME

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(Presented by: Michael K. Holland, PhD)

The sperm surface is a dynamic microenvironment the protein components of which mediate many of the biological functions sperm fulfill. Understanding the proteins composing this microenvironment provides information important for these functions. Here we report the first detailed map of the protein components of the bovine sperm plasma membrane.

Plasma membrane fractions were prepared from Bos indicus sperm collected by electroejaculation (n=8 reps) using nitrogen cavitation followed by differential centrifugation and purification on a sucrose gradient. Two bands were collected with the lighter band showing homogenous membrane vesicles under EM whilst the heavier band consisted of a more heterogenous population of vesicles. Two markers of bovine plasma membranes, alkaline phosphatase and wheat germ agglutinin binding, showed 8.1 and 5 fold enrichment respectively. Band1 and Band II, were processed separately using the filter−aided sample preparation (FASP) and subjected to analysis using five mass spectrometry workflows. Electrospray ionisation (ESI) and matrix−assisted laser desorption/ionization (MALDI) were used in conjunction with both strong cation exchange and 1−D SDS−PAGE pre−fractionation techniques. Of the 444 proteins identified approximately two−thirds of the list were predicted to contain a signal peptide, transmembrane domain or undergo non−classical secretion.

T−complex 1 chaperones, ADAMs, solute carrier channels and members of the 26S proteasome complex were detected, as were some proteins that have previously been reported to participate in capacitation (e.g. PYY2, SPADH1), sperm−egg interaction (e.g. SPAM1, CD9, IZUMO1, ZPBP1,2, ACE) or fertility (e.g. BSP 1,3,5, CCT8, PSMA1, AK1). Another major class of the proteins identified could be grouped into fundamental metabolic processes: (i) glycolysis; (ii) energy production and (iii) signal transduction. Glycolytic enzymes may exist in complexes within the plasma membrane near the mitochondrial sheath, due to the reduced cytoplasmic volume of spermatozoa, as has previously been suggested. This may be the most efficient means to generate energy for transport systems and motility. Finally, a number of DNA/RNA binding proteins and proteins involved in chromatin metabolism were identified. This raises questions about chromatin stability and repair which warrant more attention.

SPERMATOGENESIS / STEROIDOGENESIS / TESTIS BIOLOGY

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UPREGULATION OF MIR-630 BY HEAT SHOCK LEADS TO DECREASED SOX30 EXPRESSION IN SPERMATOGENESIS

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(Presented by: Yung-Ming Lin, MD)

Introduction and Objectives: Spermatogenesis relies heavily on post−transcriptional gene regulation, and its defect could profoundly influence germ cell proliferation and differentiation, leading to spermatogenic failure. MicroRNAs (miRNAs) are short non−coding RNA molecules which

play regulatory roles in repressing translation or cleaving RNA transcripts. By using microRNA microarray, we have identified human microRNA 630 (miR-630) that is significantly up-expressed in testes with spermatogenic failure. This study was conducted to explore the upstream regulator and putative targets of miR-630 and to unravel the biological function of miR-630 in human spermatogenesis.

Methods: The upstream regulator and downstream target of miR-630 were identified in silico. The expression profile of selected miR-630 target gene was determined by qRT-PCR and quantitative immunohistochemistry analysis. The interaction between miR-630 and target was studied by correlation assay, luciferase assay and gene-overexpression assay.

Results Obtained: A total of 8 spermatogenesis-related targets were identified including HMGCR, SPAM1, SPAG1, CRISP3, PSME4, TAC3, GNAS and SOX30. Of the eight targets, miR-630 significantly decreased the SOX30 3' untranslated region activity in HeLa cells, PC-3 cells and HEK293 cells by luciferase assay. Addition of miR-630 mimics attenuated the SOX30 expression at protein levels. SOX30 is expressed in the Sertoli cells of human testis, and significantly decreased SOX30 protein expression was noted in the testes with spermatogenic failure. Inverse correlation between miR-630 transcript levels and SOX30 protein expression levels was observed in the testes of infertile men. Three main transcriptional factors, including HSF, ER and SOX9, are predicted, and heat shock was found to significantly upregulate miR-630 expression and downregulate SOX30 protein expression.

Conclusions: Our results show that upregulation of miR-630 by heat shock leads to decreased SOX30 expression in spermatogenesis. Given that SOX30 has been shown to play important role in male germ line differentiation, our results further link the molecular processes of heat shock and spermatogenic failure.

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INITIAL CHARACTERIZATION OFTRIOSEPHOSPHATE ISOMERASE ISOZYMES IN MALE MOUSE GERM CELLS

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(Presented by: George Gerton, PhD)

Glycolysis converts glucose into pyruvate via series of reactions by ten enzymes, yielding two ATPs from a single glucose molecule, and is a critical source of energy for motility in mouse sperm. During glycolysis, aldolase converts fructose 1,6-diphosphate to glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). However, only GAP is the direct substrate for the GAP dehydrogenase; triosephosphate isomerase (TPI) is required to convert DHAP to GAP. Although TPI in the male germline has been studied in the human and rat, little is known about the mouse enzyme. We previously reported that mouse TPI was present in the sperm flagellar accessory structures and was also identified as a cauda epididymal sperm-differential protein. In this study, we have investigated mouse TPI further. By immunoblotting, we detected two male germline-specific Mr ~33,400 and ~30,800 TPI bands as well as the somatic-type Mr ~27,700 band. Although all three bands were observed in germ cells from pachytene spermatocytes through to condensing spermatids, somatic-type TPI disappeared from sperm during epididymal maturation. In vitro dephosphorylation analysis suggested that the two male germline-specific TPI isoforms do not undergo phosphorylation. Also the Mr ~33,400, ~30,800, and ~27,700 TPI bands corresponded to the sizes of the proteins predicted to use the first, second and third possible initiation codons of the Tpi 1 cDNA, respectively. These results suggest that the TPI isozymes were translated using the three different initiation codons. Next, we performed immunofluorescence with methanol-permeabilized sperm and determined that TPI was specifically localized in the principal piece. The antibody staining was stronger in cauda epididymal sperm than in caput epididymal sperm, a finding consistent with the identification of TPI as a cauda epididymal sperm-differential protein. Immunofluorescence with SDS-insoluble flagellar accessory structures from sperm showed a strong signal of TPI only in the principal piece, indicating that TPI is a component of the fibrous sheath. This is consistent with the studies of other glycolytic enzymes that were found tethered to the fibrous sheath of the sperm flagellum. Immunoelectron microscopy is ongoing to confirm these results.

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LOCALIZATION OF PPARS AND SIRTUINS IN THE NORMAL MURINE/HUMAN TESTIS AND THEIR ALTERATIONS IN PATIENTS WITH INFERTILITY

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(Presented by: Yu Xiao, MSc)

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors, influencing NF-kappa B-activation and the transcription of genes encoding antioxidant enzymes (e.g. SOD2 and catalase) or proinflammatory proteins (e.g. iNOS). Moreover, PPARs also control the expression of genes of peroxisomal β-oxidation enzymes, linking lipid and ROS metabolism together. Activation of PPARs is regulated by phosphorylation and acetylation. Sirtuin 1 is the specific deacetylase, which inhibits PPAR activation. This enzyme catalyzes the deacetylation reactions in a NAD+ -dependant fashion. Sirtuin 1 deacetylates a number of other important transcriptional regulatory proteins, e.g. NF-kappa B, p53 and PGC1 α, in addition to the ones of the PPAR family. The transcription of the sirtuin I gene is inhibited in a negative feedback loop by PPARγ. To date in normal testis or testis biopsies of patients with idiopathic infertility there is no information available on PPAR and sirtuin distribution and abundance. We hypothesize that oxidative stress and concomitant alterations in the PPARγ-sirtuin I-negative feedback loop might lead to male infertility.

In our study, we investigated the localization and alterations of PPARα, β, γ, antioxidant enzymes as well as different sirtuins (SIRT I-7) in the testis of mice, humans and patients with testicular inflammation and infertility. PPARα and γ were mainly localized in Sertoli cells and Leydig cells, whereas the highest PPARβ protein amount was detected in round spermatids. In contrast, cytoplasmic/nuclear sirtuin 1 and mitochondrial sirtuins 3 and 4 were mainly present in primary spermatocytes and spermatogonia. In human a comparable distribution of the investigated proteins was found as in mice. Preliminary data suggest that in the testis of patients with inflammation and infertility a downregulation of sirtuin I might occur, in conjunction with a strong upregulation of PPARγ in the same samples, suggesting an overactivation of the PPARγ/sirtuin I-negative feedback loop in the patient testis.

abstracts full text

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CODING REGION AND 3' UTR RNA EDITING IN THE TESTIS

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(Presented by: Elizabeth Snyder, PhD)

RNA editing, the modification of a nucleotide within an mRNA, has the capacity to alter the proteome by changing both the coding potential of a given message and regulatory elements in the 3' UTR. Adenosine to inosine (A to I) editing is the most commonly observed RNA editing event in mammals and is catalyzed by the activity of double stranded RNA-specific adenosine deaminase (AD) enzymes (ADARs). One potential regulator of A to I editing is adenosine deaminase domain containing 1 (ADAD1, formally known as TENR). Due to an amino acid change in ADAD1's AD domain, it is likely catalytically inactive while still maintaining the ability to bind double stranded RNA (dsRNA). Notably, global loss of Adad1 results in male infertility, due to failures in spermiogenesis. This observation suggests regulation of RNA editing may be important for male fertility. In order to assess if RNA editing occurs in the testis, the expression of RNA editing enzymes was assessed by real time RT-PCR and Western blot. Additionally, A to I editing events in the testis were computationally identified using high throughput RNA sequencing and confirmed by inosine chemical erasure (ICE). The resulting data demonstrate the testis expresses the necessary enzymes to mediate A to I RNA editing and this editing can affect both the coding capacity of targeted messages and regulatory elements within the 3' UTR. Evidence demonstrating RNA editing does occur in the testis supports the pursuit of characterizing the mechanisms and targets of RNA editing in the testis.

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UP-REGULATION OF TESTICULAR HYPOXIA-INDUCIBLE FACTOR-1α FOLLOWING LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION

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Monmouth University
(Presented by: Dharm Patel)

Bacterial and viral infections and resulting inflammation of the male reproductive tract are known to impair fertility through mechanisms that include decreased sperm mobility through the tract, blockage of the tract and reduced androgen output. Identifying molecular changes following inflammation within tissues is a topic of intense research. Hypoxia-Inducible Factor-1 (HIF-1) is a transcription factor that is considered the master regulator of hypoxia. Recent work has revealed that HIF-1 is involved in inflammatory pathways and is important for cross-talk between hypoxic and inflammatory pathways. Previously, we have shown that HIF-1 is abundantly expressed in rat Leydig cells under normoxic and hypoxic conditions. We hypothesize that HIF-1α in the rat testis is up-regulated following lipopolysaccharide (LPS)-induced inflammation. Induction of inflammation in rats was accomplished via intraperitoneal administration of LPS from E. coli and P. aeruginosa for 1, 3 and 6 hours (n = 3-5 animals/time point) at a dosage of 5 mg/kg body weight. Western Blot analysis of testicular cytoplasmic and nuclear protein extracts demonstrated an increase in HIF-1α protein. No change in HIF-1α was observed after 1 hour of E. coli LPS treatment, but a significant increase in HIF-1α protein level was observed after P. aeruginosa LPS treatment for 3 hours and E. coli LPS treatment for 6 hours. To examine potential mechanisms involved in the up-regulation of HIF-1α, DNA binding activity and protein levels of nuclear factor-kB (NF-kB), a master regulator of innate immunity and inflammation, were measured. Electromobility shift assays (EMSA) suggest a decrease in NF-kB binding activity following LPS treatment while Western Blot analysis shows no change in NF-Î°B protein levels. These results suggest that the up-regulation of HIF-1α is not regulated by NF-kB. Currently, qPCR experiments are underway to determine if the increase in HIF-1α protein levels is caused by transcriptional regulation. Further experiments will be performed to examine the mechanisms affecting levels of HIF-1α following LPS-induced inflammation. The overall objective of this work is to elucidate the link between hypoxic and inflammatory responses in the testis at the molecular level following inflammation. This relationship may be useful in studying disease states in which hypoxia and inflammation are features of the microenvironment.

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PROTEOMIC ANALYSIS OF GENE EXPRESSION IN GOLGA3<REPRO27> MALE INFERTILITY MICE

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(Presented by: Rahul Sigdel, BS)

Golgin subfamily A member 3 protein, GOLGA3, is a Golgi apparatus associated protein required for spermatogenesis. The absence of GOLGA3 protein in Golga3repro27 mice leads to a partial block in meiosis, germ cell death, and abnormal formation of the sperm head and tail causing complete male infertility. GOLGA3 is implicated in protein targeting, vesicular trafficking, and apoptosis but its role in sperm development has not been determined. The objective of this study was to use a proteomics approach to identify proteins whose expression pattems have changed in Golga3repro27 mice to unravel the role of GOLGA3 in spermatogenesis. 2-D gel electrophoresis was performed on testicular protein (14 dpp) isolated from C3Fe.B6-Golga3repro27 mutant mice (congenic strain that is ~99.8% C3HeB/FeJ; n=3, pooled) and C3HeB/FeJ controls (n=3, pooled). 15 protein spots showed lower or absent expression in mutants compared to controls. The spots were excised, subjected to in-gel trypsin digestion, and analyzed by MALDI mass spectrometry including peptide mass fingerprinting and MS/MS analysis on the five most intense peptides in each protein spot. Differentially-expressed proteins include valosin containing protein (VCP), caldesmon 1 (CALD1), tubulin beta-5 chain (TUBB5), and transferrin (TRF). VCP is presumed to act as cellular sensor protein that detects abnormal protein accumulation and promotes its degradation through Endoplasmic-reticulum-associated protein degradation (ERAD). CALD1 is an actin-linked regulatory protein responsible for actin-myosin interaction and stabilizing actin filaments. TUBB5 is a regulatory cytoskeletal protein that has a GTP binding domain. TRF plays a critical role transporting iron in Sertoli cells. Confirmation of these proteins with an additional biological replicate and future experimentation will lead to an increased understanding of the role of GOLGA3 in spermatogenesis.

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EXPRESSION OF HEDGEHOG PATHWAY REGULATORS IN THE ADULT TESTIS REVEALS DYNAMIC CONTROL OF SIGNALLING ACTIVITY

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(Presented by: Kate Loveland, PhD)

Background: The Hedgehog (Hh) signaling pathway is essential for normal fetal testis development, and recent studies suggest Hh activity is required for adult spermatogenesis in rodents. Our previous work identified adult Sertoli cells as the source of the sole testicular Hh ligand, Desert Hedgehog (Dhh), and receptors, signal transducers and transcription factors for this pathway are synthesized in male germ cells in a developmentally–regulated manner. Three Gli transcription factors mediate transcriptional outcomes from Hh signalling: Gli1, which activates Hh target genes and is itself an Hh target, Gli2, typically an activator, and Gli3, which represses Hh target genes. A key outcome from our earlier work was the discovery that SuFu, a negative regulator of Hh activity which prevents Gli1 from entering the nucleus, is produced exclusively in elongating spermatids (EStids). This suggested that Hh signalling is switched off in late spermiogenesis. Further immunohistochemical (IHC) studies and examination of how Gli proteins gain nuclear access reveal several potential mechanisms for Hh activity control that we propose operate at specific phases of spermatogenesis.
Materials and Methods: IHC was performed with antibodies to Gli2 (Abcam) and Gli3 (Santa Cruz) on Bouin's fixed, paraffin–embedded sections of mouse and human adult testes obtained with institutional Animal and Human Ethics Committee approval. Recombinant mouse proteins encoding Gli1 constructs, SuFu and importin proteins were produced in bacteria, purified and then tested for binding in ALPHAScreen competition assays.
Results: The Gli2 signal was predominantly nuclear in spermatocytes (Scytes) and round spermatids (RStids) of adult mouse and human testes, while only cytoplasmic Gli2 was detected in EStids. In contrast, Gli3 was nuclear in spermatogonia and RStids, but cytoplasmic in Scytes and EStids. High affinity binding between the nuclear transport protein, importin beta1 and Gli1, was effectively competed by a recombinant SuFu construct.
Discussion: The complimentary expression profiles of Gli2 and Gli3 in adult testes extends published findings that demonstrate Hh signalling is active in spermatocytes and round spermatids but down–regulated in spermatogonia, before meiotic entry, and EStids, in late spermiogenesis. Thus regulated Gli protein synthesis and competition for nuclear transport machinery may control Hh activity at crucial phases of spermatogenesis.

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