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ANDROLOGY

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Abstracts from the 39th American Society of Andrology Annual Meeting

5 – 8 April 2014

Atlanta, Georgia

The merged journal of the American Society of Andrology and the European Academy of Andrology, now including the former *International Journal of Andrology and Journal of Andrology*

WILEY Blackwell

American Society of Andrology

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ANDROLOGY

APRIL 2014 VOLUME 2 SUPPLEMENT 1

Abstracts from the 39th American Society of Andrology Annual Meeting
5 – 8 April 2014
Atlanta, Georgia

WILEY Blackwell

SCHEDULE AT A GLANCE

ASA 39th Annual Conference
April 5 – 8, 2014

ASA Basic Science Workshop
April 5, 2014

Andrology Lab Workshop
April 5, 2014

ASA Special Symposium
April 5, 2014

ASA 39th Annual Conference
"Andrology: Where Are We and Where Are We Going?"
April 5 – 8, 2014
InterContinental Buckhead Atlanta
Atlanta, Georgia
Program Chairs: Robert E. Brannigan, MD and Barry R. Zirkin, MD
Location: Windsor Ballroom C-E

FRIDAY, APRIL 4, 2014

2:00 p.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

SATURDAY, APRIL 5, 2014

7:30 a.m. – 7:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

4:00 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

8:30 a.m. - 4:00 p.m. **ASA Basic Science Workshop**
(See pg. 27 for full schedule)

9:00 a.m. - 5:00 p.m. **ASA Andrology Lab Workshop**
(See pg. 28 for full schedule)

1:00 p.m. - 5:15 p.m. **ASA Special Symposium**
(See pg. 29 for full schedule)

5:30 p.m. – 5:40 p.m. **Welcome and Opening Remarks**

5:40 p.m. – 6:00 p.m. **Updates from NICHD & NIEHS: Where Are We and Where Are We Going?**
Stuart B. Moss, PhD
NICHD
Thaddeus T. Schug, PhD
NIEHS

6:00 p.m. – 6:20 p.m. **Distinguished Andrologist Award**

6:20 p.m. – 6:30 p.m. **Centers for Disease Control and Prevention Welcomes ASA to Atlanta**
Hubert Vesper, PhD
National Center for Environmental Health

6:30 p.m. – 7:30 p.m. **EMIL STEINBERGER MEMORIAL LECTURE**
iPS Cell Technology and Disease Research: Issues to be Resolved
Rudolf Jaenisch, MD
Massachusetts Institute of Technology
(Introduced by Erwin Goldberg, PhD)

7:30 p.m. – 9:30 p.m. **Welcome Reception**
Location: Windsor Ballroom AB

SUNDAY, APRIL 6, 2014

6:30 a.m. – 8:00 a.m. **Past President's Breakfast**
Location: Trippe 1

6:30 a.m. – 6:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

7:00 a.m. – 4:00 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

7:00 a.m. – 8:00 a.m. **Continental Breakfast in Exhibit Hall**
Location: Windsor Ballroom AB

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
Controversies in Vasectomy and Vasectomy Reversal
Jay I. Sandlow, MD
Medical College of Wisconsin
(Introduced by Robert E. Brannigan, MD)

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

9:15 a.m. – 10:45 a.m. **SYMPOSIUM I – Stem Cells in the Male Reproductive Tract**
Co-chairs: Marie-Claude Hofmann, PhD; Makoto Nagano, PhD, DVM

SCHEDULE AT A GLANCE

SUNDAY, APRIL 6, 2014 ***(continued)***

Unraveling Signaling Pathways Controlling Gonocyte Differentiation
Martine Culty, PhD
McGill University

Regulation of Spermatogonial Stem Cells in the Adult Testis
William Wright, PhD
Johns Hopkins Bloomberg School of Public Health

Human and Non-Human Primate Stem Cells
Kyle Orwig, PhD
University of Pittsburgh School of Medicine

10:45 a.m. – 11:00 a.m. **Break**
Location: Windsor Ballroom AB

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

12:30 p.m. – 2:00 p.m. **MENTORING LUNCHEON SPONSORED BY THE DIVERSITY AND TRAINEE AFFAIRS COMMITTEES**
Embarking on a Scientific Career: Combining Administrative, Teaching and Clinical Responsibilities
Location: Trippe 1
William J. Bremner, MD, PhD
University of Washington
(Introduced by Peter Liu, MBBS, PhD)
**Not included in registration fee; ticket required*

12:30 p.m. – 2:00 p.m. **Editorial Board Luncheon**

12:30 p.m. – 2:00 p.m. **Lunch On Your Own**

CONCURRENT ORAL SESSIONS

2:00 p.m. – 3:30 p.m. **Oral Session I: Molecular and Environmental Regulation of Male Reproductive Health**
Location: Windsor C - E
Moderators: Kate Loveland, PhD; Jacquetta M. Trasler, MD, PhD

2:00 p.m. – 3:30 p.m. **Oral Session II: Human Spermatogenesis: Novel Findings in 2014**
Location: Hope
Moderators: Dolores J. Lamb, PhD; Kyle Orwig, PhD

3:30 p.m. – 4:00 p.m. **Break**
Location: Windsor AB

4:00 p.m. – 4:45 p.m. **LECTURE I**
What's Good for the Spermatogonial Stem Cell May Be Bad for the Offspring: Advantageous Mutations that Increase the Incidence of Human Disease
Norman Arnheim, PhD
University of Southern California
(Introduced by Mary A. Handel, PhD)

4:45 p.m. – 5:30 p.m. **LECTURE II**
Novel Spermatogenic Pathways and Male Contraception
Martin M. Matzuk, MD, PhD
Baylor College of Medicine
(*Introduced by Jacquetta M. Trasler, MD, PhD*)

5:30 p.m. – 6:15 p.m. **SYMPOSIUM – Updates from the Centers for Disease Control and Prevention: Progress in Male Reproductive Health**
Moderator: Steven M. Schrader, PhD
National Institute for Occupational Safety and Health, CDC

Insights Gained from CDC Surveys and Initiatives
Lee Warner, PhD
National Center for Chronic Disease Prevention and Health Promotion, CDC

CDC's Hormone Standardization Program: A Focus on Testosterone
Hubert Vesper, PhD
National Center for Environment Health, CDC

SCHEDULE AT A GLANCE

Discussion: Potential Collaboration with Academic Programs and National Organizations

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: Windsor Garden

MONDAY, APRIL 7, 2014

7:00 a.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

7:00 a.m. – 8:00 a.m. **Continental Breakfast**
Location: Windsor Foyer

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Hormone Signaling and Reprogramming in Human Prostate Stem Cells
Gail S. Prins, PhD
University of Illinois at Chicago
(*Introduced by Donna L. Vogel, MD, PhD)*

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

9:15 a.m. – 10:15 a.m. **SYMPOSIUM II – Would You Give This Man Testosterone? Case-Based Discussions**
Moderators: Christina Wang, MD
Stephanie T. Page, MD, PhD

J. Lisa Tenover, MD, PhD
VA Palo Alto Health Care System
Peter N. Schlegel, MD
The New York Weill/Cornell Medical Ctr.

10:15 a.m. – 10:30 a.m. **Break**
Location: Windsor Foyer

10:30 a.m. – 11:15 a.m. **DIVERSITY LECTURE**
Disparities in Men's Health: The Role of the Primary Care Physician
Charles S. Modlin, MD
Cleveland Clinic Foundation, Minority Men's Health Center
(Introduced by George L. Gerton, PhD)

11:15 a.m. – 12:30 p.m. **Poster Session II**
Location: Venetian

12:30 p.m. – 1:45 p.m. **WOMEN IN ANDROLOGY LUNCHEON AND DISCUSSION**
What Successful Women Do Differently: Learning To Embrace Failure and To Take Risks
Location: Trippe
Moderator: Sophie La Salle, PhD
**Not included in registration; ticket required*

1:45 p.m. – 3:15 p.m. **SYMPOSIUM III Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility**
Co-Chairs: Gail A. Cornwall, PhD
Kenneth P. Roberts, PhD

Qualitative and Quantitative Aspects of the Hormonal Control of Spermatogenesis Revisited
Ilpo Huhtaniemi, MD, PhD, FMed Sci
Imperial College, London
Ca2+ and cAMP Signaling Crosstalk During Sperm Capacitation
Pablo E. Visconti, PhD
University of Massachusetts

Aging Affects Germ Cells: From Genes to Fertility
Bernard Robaire, PhD
McGill University

3:15 p.m. – 3:30 p.m. **Break**
Location: Windsor Foyer

3:30 p.m. – 4:15 p.m. **LECTURE III:**
The Stress Hormone Corticotropin-Releasing Factor Acts in the Brain and the Testes to Regulate Testosterone Secretion
Catherine Rivier, PhD
The Salk Institute for Biological Studies
(Introduced by Vassilios Papadopoulos, PhD)

4:15 p.m. – 5:00 p.m. **LECTURE IV:**
Pharmacological Regulation of Steroid Biosynthesis: From Testis to Brain
Vassilios Papadopoulos, PhD
The Research Institute of the Mc Gill University Health Centre
(Introduced by Catherine Rivier, PhD)

5:00 p.m. – 6:00 p.m. **ASA Business Meeting**
Outstanding Trainee Investigator and Trainee Awards

7:30 p.m. – 11:00 p.m. **Annual Banquet**
Location: Atlanta Event Center at Opera
Buses depart from hotel lobby starting at 6:45 p.m.
**Not included in registration fee; ticket required*

TUESDAY, APRIL 8, 2014

7:00 a.m. – 8:00 a.m. **2015 Program Committee Meeting**
Location: Hope

7:00 a.m. – 12:00 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

7:00 a.m. – 8:00 a.m. **Continental Breakfast**
Location: Windsor Foyer

8:00 a.m. – 9:15 a.m. **SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?**
Moderators: Gail S. Prins, PhD
Arthur L. Burnett, II, MD

William J. Catalona, MD
Northwestern University
Timothy Wilt, MD
University of Minnesota School of Medicine
Herbert B. Carter, MD
Johns Hopkins Hospital

9:15 a.m. – 10:15 a.m. **INTERNATIONAL LECTURE:**
Pharmacogenetics of FSH
Manuela Simoni, MD, PhD
University of Modena and Reggio Emilia, Italy
(Introduced by Patricia S. Cuasnicu, PhD)

10:15 a.m. – 10:30 a.m. **Break**
Location: Windsor Foyer

10:30 a.m. – 12:00 p.m. **SYMPOSIUM V – Innovations in Male Environmental Health Protection**
Co-Chairs: Sally Perreault Darney, PhD
Bernard Robaire, PhD

Revolution in Toxicity Testing and Risk Prediction for Chemicals in the Environment
Thomas Knudsen, PhD
US Environmental Protection Agency

Response of Human Fetal Testis Xenotransplants to Environmental Toxicants: Implications for Risk Assessment
Kim Boekelheide, MD, PhD
Brown University

Translation of the Science in Male Reproductive and Environmental Health for Evidence-Based Decisions by Clinicians, Regulators and the Public
Paula I. Johnson, PhD
University of California, San Francisco

MEETING ADJOURNED

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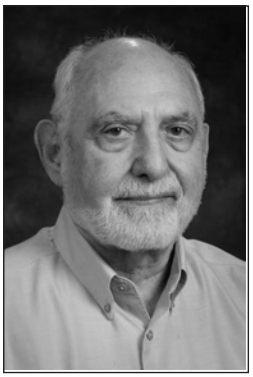
PRESIDENT'S WELCOME



It is my pleasure to welcome everyone to the 39th Annual Meeting of the American Society of Andrology, entitled "Andrology: Where Are We and Where Are We Going?" The query is even more compelling in this era of constraints on funding for basic research. Limitations on basic research impact not only men's health, but the health and well-being of males of all species. We will hear about the state of the funding landscape at the very start of our sessions. We are meeting in Atlanta, Georgia which has been described as the "capital city of the Southeast, a city of the future with strong ties to its past". I like to think of ASA in those terms, a society of the future with a distinguished heritage of past achievements in animal and men's health. This is consistent with the mission of the Society, to advance discovery and education in male reproductive health through the integration of basic and clinical sciences and scientists. This mission is clearly the intent of the 39th Annual Meeting. The Program Co-Chairs, Barry Zirkin, PhD (Johns Hopkins Bloomberg School of Public Health) and Robert Brannigan, MD (Northwestern University) have assembled cutting edge and timely symposia and lectures blending basic and clinical research. The Program is seamless in that respect, which means that we will not find it easy to miss a session based on its being labeled "clinical" or "basic". The Program begins at the beginning with the cell. The Emil Steinberger Memorial Lecture, "iPS Cell Technology and Disease Research: Issues to be Resolved", will be presented by a pioneer in stem cell biology, Professor Rudolf Jaenisch (MIT). There are Major Symposia on stem cells in the male reproductive tract; testosterone replacement; spermatogenesis, sperm maturation and fertility, as well as new thinking about PSA and prostate cancer; and innovations in male environmental health protection.

Major Lectures on stem cells in relation to the incidence of human disease; novel spermatogenic pathways with relevance to male contraception; and the regulation of testosterone secretion by factors in the brain and testis are prominent in this program. The annual Women in Andrology Lecture will focus on hormone signaling and reprogramming in human prostate stem cells. Differences of opinion that are necessary for good science will be reflected in the AUA Lecture, "Controversies in Vasectomy and Vasectomy Reversal"; a debate on PSA screening and Prostate Cancer; and T therapy in clinical practice. The Women in Andrology Luncheon will focus on the qualities of successful women in science. It, therefore, is not surprising that three women are the recipients of this year's ASA awards: Gail Prins will be honored as the 2014 Distinguished Andrologist; Sara Kimmins will receive the Young Andrololologist Award; and Susan Rothman will receive the Distinguished Andrologist Award.

An important function of ASA is engaging students in our endeavors. A Mentoring Luncheon is scheduled that will examine what it takes to embark on a scientific career. Our membership committee is working diligently to recruit more student members to ASA so please encourage your students to join.

Atlanta is the home of the Centers for Disease Control and Prevention's (CDC). ASA will expand its association with CDC by hosting a special symposium dealing with the role of the agency in research areas of relevance to Andrology.
There is a critical need to raise the awareness in our society of men's health issues by publicizing our work as a way to promote the importance of investing in basic and applied research in the United States. This cannot be over-emphasized. We have established a website, "Andrology America," which we hope will serve this purpose by making it somewhat easier to answer the question, "What is Andrology?"

Be sure to attend the Banquet and Dance which will be held at the Atlanta Event Center at Opera, the premier event center in Atlanta, Georgia. Housed in a building that was originally constructed as an opera house in the early roaring 1920s, Opera is known among the most stylish and well-located event facilities throughout the Southeast.

Finally, I am honored to have had the privilege of serving this past year as president of ASA. I am pleased to report that the society continues to be a viable and important component of the American scientific community. I am extremely proud of the Program for the 39th Annual Meeting of ASA, and thank you all for joining me in Atlanta.

Erwin Goldberg, PhD
President, American Society of Andrology

PAST PRESIDENTS OF THE AMERICAN SOCIETY OF ANDROLOGY

1975-1977	Emil Steinberger*
1977-1978	Don W. Fawcett*
1978-1979	C. Alvin Paulsen*
1979-1980	Nancy J. Alexander
1980-1981	Philip Troen
1981-1982	Richard M. Harrison
1982-1983	Richard J. Sherins
1983-1984	Andrzej Bartke
1984-1985	Rudi Ansbacher
1985-1986	Anna Steinberger
1986-1987	William D. Odell
1987-1988	Larry L. Ewing*
1988-1989	C. Wayne Bardin
1989-1990	Rupert Amann
1990-1991	Howard Nankin
1991-1992	David W. Hamilton
1992-1993	Ronald S. Swerdloff
1993-1994	Bernard Robaire
1994-1995	Glenn R. Cunningham
1995-1996	Marie-Claire Orgebin-Crist
1996-1997	Arnold M. Belker

PAST PRESIDENTS (CONTINUED)

1997-1998	Terry T. Turner
1998-1999	Richard V. Clark
1999-2000	Barry T. Hinton
2000-2001	J. Lisa Tenover
2001-2002	Barry R. Zirkin
2002-2003	Jon L. Pryor
2003-2004	Gail S. Prins
2004-2005	William J. Bremner
2005-2006	Sally Perreault Darney
2006-2007	Christina Wang
2007-2008	Terry R. Brown
2008-2009	Wayne J.G. Hellstrom
2009-2010	Dolores J. Lamb
2010-2011	Paul J. Turek
2011-2012	Gail A. Cornwall, PhD
2012-2013	Donna L. Vogel, MD, PhD

**Deceased*

AMERICAN SOCIETY OF ANDROLOGY

OFFICERS

President	Erwin Goldberg, PhD
Vice President	Jay I. Sandlow, MD
Secretary	Jacquetta M. Trasler, MD, PhD
Treasurer	Rex A. Hess, MS, PhD
Past President	Donna L. Vogel, MD, PhD

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Barry R. Zirkin, PhD; Baltimore, MD
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Marvin L. Meistrich, PhD; Houston, TX
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Liaison Committee
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Membership Committee
Alan Diekman, PhD; Little Rock, AR
Nominating Committee
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Publications and Communications Committee
Jacques J. Tremblay, PhD; Quebec City, QC Canada
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Allen D. Seftel, MD, FACS; Camden, NJ (Co-Chair)
Trainee Affairs
Peter Liu, MBBS, PhD; Torrance, CA
George L. Gerton, PhD; Philadelphia, PA (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 MEETING INFORMATION

Located in the north-west region of the state, Atlanta is not only the capital, but also Georgia's largest city, and operates as the main transportation hub of the southeast. From General William T. Sherman's March to the Sea during the Civil War to the founding of The Coca-Cola Company, and most memorably, as the birthplace of Martin Luther King, Jr. and a major organizing city in the Civil Rights Movement, Atlanta provides a rich historical culture waiting to be explored.

ATTRACTIONS
There are endless options of exciting activities and attractions in Atlanta. Visit the World of Coca-Cola for an educationally delicious look into the world's most famous soft drink where you will have the opportunity to learn about the history, sample over 60 different flavors of Coca-Cola from around the world and more. Delve into the legacy of the leader of the Civil Rights Movement at the Martin Luther King, Jr. National Historic Site. Throughout the site you will find his original gravesite, current tomb, boyhood home and much more. Check out the Georgia Aquarium, the world's largest aquarium. It features tens of thousands of animals of over 500 species, including displays with whale sharks and manta rays, and beluga whales, as well as a must-see dolphin gallery.

SHOPPING
Atlanta offers it all, from boutiques to outlet centers to art galleries and antiques, making the shopping here always exciting. Visit Atlanta's Buckhead District, a chic neighborhood with luxury shopping in sought after destinations, such as Lenox Square and Phipps Plaza. Explore Atlanta's neighborhoods, such as Decatur and Bennett Street, to browse through an array of charming boutiques selling everything from clothing and custom made jewelry to furniture and works of art.

DINING/NIGHTLIFE
The Atlanta dining scene offers a melting pot of choices from the traditional Atlanta soul food, to more refined and original recipes that are sure to make any foodie excited. For instance, head over to the Paschal's where they have been serving up southern cooking from fried chicken and fried green tomatoes to barbecue ribs since 1947, or visit Anis Café & Bistro for their signature mussels and other indulgent dishes.
After dinner, a diverse selection of nightlife awaits in Atlanta, with laid back, chic and trendy options to choose from. Step into Buckhead Bottle Bar, a stylish restaurant that serves up original cocktails, and is open late, fit for an evening of dining, drinks and dancing. Atlanta also offers a variety of theatre and production options from outdoors at the Chastain Park Amphitheater or the Verizon Wireless Amphitheatre, to Broadway's best at Fox Theatre and many more!

WEATHER
Atlanta has a warm, humid climate with April characterized by rising temperatures. The average temperature for early April is a high of 70 degrees Fahrenheit and a low of 50 degrees Fahrenheit.

OUTDOOR RECREATION
Explore Stone Mountain Park where exciting adventures and historical sights are in store. The park features a Geyser Tower, a SkyHike, which is the nation's largest adventure course, scenic rides around the mountain and much more. Stop by the foot of the mountain to find the Stone Mountain Golf Club for a relaxing and picturesque game of golf. Housing interesting animals from around the world, Zoo Atlanta is the place to go to see animal shows and fascinating exhibits, and enjoy a picnic in the park.

ARTS & CULTURE
With 11,000 pieces from around the world, Atlanta's High Museum of Art is not lacking in range. Their main collection features work from Monet, Tournier, Tiepolo and Ernst, among many other notable artists. Browse through Atlanta's unique neighborhood galleries, which include the inventive, Museum of Design Atlanta (MODA). Or check out the Atlanta Symphony Orchestra, a Grammy Award-winning orchestra that is sure to entertain and impress.

Registration/Information Desk Hours are as follows:

Friday, April 4, 2014:
2:00 p.m. – 6:00 p.m.

Saturday, April 5, 2014:
7:30 a.m. – 7:30 p.m.

Sunday, April 6, 2014:
6:30 a.m. – 6:30 p.m.

Monday, April 7, 2014:
7:00 a.m. – 6:00 p.m.

Tuesday, April 8, 2014:
7:00 a.m. – 12:00 p.m.

Exhibit Hall Hours are as follows:

Saturday, April 13, 2013:
4:00 p.m. – 9:30 p.m.

Sunday, April 14, 2013:
7:00 a.m. – 4:00 p.m.

HOTEL INFORMATION

The American Society of Andrology 2014 Annual Conference will be held at the beautiful InterContinental Buckhead Atlanta in Atlanta, Georgia where special room rates have been arranged for meeting attendees.

InterContinental Buckhead Atlanta
3315 Peachtree Road NE
Atlanta, GA 30326
Main: (404) 946-9000
Fax: (404) 521-1327
Website: http://www.ichotelsgroup.com/intercontinental/en/gb/locations/atlanta

Room Rate: $175.00
Hotel Deadline: March 14, 2014
Reservations: (877) 422-8254

Room Rate
ASA has negotiated a discounted rate of $175.00 plus tax (currently 16%) at the InterContinental Buckhead Atlanta Hotel. Additional charges for people over 17 years of age is $25.00 per person per night.

Hotel Deadline
The deadline to receive the ASA group rate is March 14, 2014. ***ASA encourages you to make your reservation early, as the hotel and discount block may sellout before this date. After this date, reservations will be accepted based on availability and higher rates may apply.***

Reservations
Attendees are responsible for making their reservations by calling the hotel at (877) 422-8254. Please reference the ASA to receive the discounted rate.

Hotel Deposit and Cancellation Policy
A credit card is required for a reservation guarantee. These deposits are fully refundable if the hotel is notified 24-hours prior to arrival and a cancellation number is obtained.

TRAVEL & TRANSPORTATION

Airport Information
Hartsfield-Jackson Atlanta International Airport is approximately 17 miles from the InterContinental Buckhead Atlanta Hotel or 30 minutes by car.

Taxi Cab Services
Several taxi companies operate at the Hartsfield-Jackson Atlanta International Airport:

Atlanta Checker Cab Company:	(404) 351-1111
A&B Taxi:	(770) 471-6646
Atlanta Lenox Taxi:	(404) 872-2600

Rental Car Information
Avis® Rent-A-Car is the official rental car company for the ASA Annual Conference. For reservations, please call (800) 331-1600, and use the code "J901055" to receive the discounted rates.

Public Transportation
Hartsfield-Jackson Atlanta International Airport offers easy access to the Metropolitan Atlanta Transit Authority System. From Hartsfield-Jackson Atlanta International Airport, a one-way fare to Buckhead costs $2.50. Please visit the following link for detailed directions: http://www.itsmarta.com/

Parking
The InterContinental Buckhead Atlanta Hotel offers self parking for $22.00 per day and valet parking for $32.00 per day. Please note that rates are subject to change.

SPECIAL EVENTS

Laboratory Science Forum Luncheon
"Kinetic Vitrification: Some Basics and Applications in Andrology Labs"
Date: Saturday, April 5, 2014
Time: 12:00 p.m. – 1:00 p.m.
Location: Trippe 1
In this year's LSF Luncheon, Dr. Igor Katkov will provide an overview of gamete cryopreservation from the perspective of vitrification. Following a review of the basics of cryopreservation, kinetic vitrification will be discussed with special emphasis on basic and emerging applications and techniques. In addition to providing a review of or first exposure to the principles of cryopreservation, this presentation will also address the principles and promise of vitrification. We look forward to your attending this cool presentation.
Cost: One ticket is included with Andrology Laboratory Workshop (ALW) registration; $35.00 for non-ALW registrants. Please sign up for this event on the ASA registration form.

Welcome Reception
Date: Saturday, April 5, 2014
Time: 7:30 p.m. – 9:30 p.m.
Location: Windsor Ballroom AB
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 Andrology Lab Workshop.
Dress: 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.

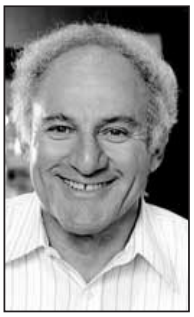
Mentoring Luncheon Sponsored by the Diversity and Trainee Affairs Committees
"Embarking on a Scientific Career: Combining Administrative, Teaching and Clinical Responsibilities"
Date: Sunday, April 6, 2014
Time: 12:30 p.m. – 2:00 p.m.
Location: Trippe 2
Dr. Bremner is a recipient of the Distinguished Andrologist Award from the ASA, and has mentored many junior faculty. He is an active clinician, chair of a large department of medicine and principal investigator of a NICHD Male Contraception Research Center Program grant. He will share his insights into how to combine administrative, teaching and clinical responsibilities whilst embarking on a research career.
Speaker: William J. Bremner, MD, PhD
Cost: $10.00 for trainees, $35.00 for non-trainees. Please sign up for this event on the registration form.

Trainee Forum and Mixer
Date: Sunday, April 6, 2014
Time: 6:30 p.m. – 8:30 p.m.
Location: Windsor Garden
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 postdoctoral fellowship 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
"What Successful Women Do Differently: Learning to Embrace Failure and to Take Risks"
Date: Monday, April 7, 2014
Time: 12:30 p.m. – 1:45 p.m.
Location: Trippe
Host: Sophie La Salle, PhD
Cost: $25.00 for trainees, $35.00 for non-trainees. Please sign up for this event on the registration form.
The ASA is rich with accomplished women andrologists thriving in a variety of positions. What is their secret? Although everyone has their own definition of success, most successful women share common traits. Please join us as we discuss the qualities and approaches that lead to our success. Time will also allow for networking with fellow women in andrology.

Annual Banquet
Date: Monday, April 7, 2014
Time: 7:30 p.m. – 11:00 p.m.
Location: Atlanta Event Center at Opera
Cost: $75.00 per person, $35.00 for trainees. Includes dinner and entertainment. Please sign up for this event on the registration form.
The Annual Banquet and Dance will be held at the Atlanta Event Center at Opera. This eclectic building was originally constructed as an opera house in the roaring 1920s and features ornate designs that create a luxurious atmosphere perfect for the night's event.

MESSAGE FROM THE PROGRAM CO-CHAIRS

Welcome to Atlanta for the 39th Annual Meeting of the American Society of Andrology. The theme of this year's meeting is "Andrology: Where Are We and Where Are We Going?" Our objective was to put together cutting edge symposia and lectures that blend basic with clinical research and that look ahead. We realized that success in doing this required bringing together outstanding scientific leaders, both MD and PhD, with accomplishment and vision. We're sure you will agree that the speakers have "been there/done that," and that all are well positioned to discuss where we have been and where we are (or should be) going!

The Emil Steinberger Memorial Lecture, which kicks off the 2014 meeting, will be delivered by Rudolf Jaenisch, PhD of MIT and the Whitehead Institute. Dr. Jaeinsch's talk is entitled "iPS Cell Technology and Disease Research: Issues to be Resolved." Dr. Jaenisch is a Founding Member of the Whitehead Institute. His research has focused on understanding epigenetic regulation of gene expression, and this work has led to major advances in our understanding of embryonic stem cells and induced pluripotent stem (IPS) cells. He has coauthored more than 375 research papers and has received numerous prizes and recognitions, including an appointment to the National Academy of Sciences in 2003. The 2014 AUA lecturer will be Jay Sandlow, MD, Director of Male Infertility at the Medical College of Wisconsin. Dr. Sandlow will speak on "Controversies in Vasectomy and Vasectomy Reversal. The Impact of Male Infertility on Men's Health: Is There a Relationship?" This is in keeping with Dr. Sandlow's long-standing interests in the basis for and treatment of male infertility, and in diseases that some of these men incur.

Five major symposia will follow these talks, each with significant translational implications. Symposium I, entitled Stem Cells in the Male Reproductive Tract, will involve a series of talks to be presented by Martine Culty, PhD (Unraveling Signaling Pathways Controlling Gonocyte Differentiation), William Wright, PhD (Regulation of Spermatogonial Stem Cells in the Adult Testis) and Kyle Orwig, PhD (Human and Non-Human Primate Stem Cells). Symposium II, entitled Would You Give This Man Testosterone? Case-Based Discussion, will be a discussion/debate by J. Lisa Tenover, MD, PhD and Peter Schlegel, MD on an issue, testosterone replacement, that has become extremely controversial. Symposium III, entitled Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility, will consist of a series of talks presented by Ilpo Huhtaniemi, PhD (Qualitative and Quantitative Aspects of the Hormonal Control of Spermatogenesis Revisited), Pablo Visconti, PhD (Ca2+ and cAMP Signaling Crosstalk During Sperm Capacitation) and Bernard Robaire, PhD (Aging Affects Germ Cells from Genes to Fertility). Symposium IV, entitled PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?, will be a debate by William Catalona, MD, Timothy Wilt, MD and Bal Carter, MD. This, too, is a "hot topic" that is controversial and extremely important. ASA's annual meeting, with its basic scientists and clinicians sitting in the same room, represents an exceptionally appropriate form in which to discuss/debate the issue. Symposium V, entitled Innovations in Male Environmental Health Protection, will consist of talks presented by Thomas Knudsen, PhD (Revolution in Toxicity Testing and Risk Prediction for Chemicals in the Environment), Kim Boekelheide, MD, PhD (Response of Human Fetal Testis Xenotransplants to Environmental Toxicants: Implications for Risk Assessment) and Paula Johnson, PhD (Translation of the Science in Male Reproductive and Environmental Health for Evidence-Based Decisions by Clinicians, Regulators and the Public).

There also will be a series of major lectures. This include the Women in Andrology Lecture by this year's Distinguished Andrologist awardee, Gail Prins, PhD, entitled Hormone Signaling and Reprogramming in Human Prostate Stem Cells; the International Lecture by Manuela Simoni, MD, PhD, entitled Pharmacogenetics of FSH; and a new Diversity Lecture on Disparities in Men's Health by Charles Modlin, PhD entitled The Role of the Primary Care Physician. Dr. William Bremner, MD, PhD will lecture on Embarking on a Scientific Career: Combining Administrative, Teaching and Clinical Responsibilities in the context of a Mentoring Luncheon sponsored by the Diversity and Trainee Affairs Committees. Additionally, there will state-of-the art major lectures by Norman Arnheim, PhD, entitled What's Good for the Spermatogonial Stem Cell May Be Bad for the Offspring: Advantageous Mutations that Increase the Incidence of Human Disease; Martin Matzuk, MD, PhD entitled Novel Spermatogenic Pathways and Male Contraception; Catherine Rivier, PhD, entitled The Stress Hormone Corticotropin-Releasing Factor Acts in the Brain and the Testes to Regulate Testosterone Secretion; and Vassilios Papadopoulos, PhD, entitled Pharmacological Regulation of Steroid Biosynthesis: From Testis to Brain.

An important feature of our annual meetings are the platform and poster sessions, this year's drawn from the over 150 submitted abstracts. There will be concurrent oral sessions that each will have six speakers, entitled Molecular and Environmental Regulation of Male Reproductive Health and Human Spermatogenesis: Novel Findings in 2014. Two poster sessions also will be held. Both the platform and poster sessions permit those attending the meeting 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.

This year's meeting will be significantly enhanced by colleagues from NIH (Drs. Stuart Moss and Thaddeus Schug, who will update us on where NIH funding and interests are, and where we likely are to be going; by the Centers for Disease Control and Prevention which will be integrally involved in a special symposium on Progress in Male Reproductive Health, including talks by Lee Warner, PhD (Insights Gained from CDC Surveys and Initiatives) and Hubert Vesper, PhD on CDC's Hormone Standardization Program: A Focus on Testosterone. There also will be a discussion on potential collaboration between CDC and academic programs and national organizations. The annual meeting will be preceded on Saturday, April 5 by the ASA Basic Science Workshop, entitled Assessing Male Reproductive Function in the Laboratory and chaired by Kate Loveland; the ASA Andrology Lab Workship entitled Post Vasectomy Semen Analysis: Lab Methods and Interpretation, chaired by Charles Muller; and a Special Symposium entitled Controversies in Testosterone Therapy: Cardiovascular Disease/Metabolic Syndrome, Prostate Cancer, and Fertility, chaired by Mohit Khera and Allen Seftel. Complementing these scientific activities will be events for networking, career development, discussion and socializing. These include the welcoming reception; the Women in Andrology Luncheon and Discussion (What Successful Women Do Differently: Learning to Embrace Failure and to Take Risks), moderated by Sophie La Salle; a Mentoring Luncheon sponsored by the Diversity and Trainee Affairs Committees featuring a lecture by Dr. Bremner noted above; and a trainee forum and mixer.

We thank ASA president Erv Goldberg, PhD for offering us this special opportunity to chair the organization of the 2014 annual meeting, and are grateful for the input and advice from the andrology community, and especially from our Program Committee members. We are particularly grateful to W.J. Weiser and Associates for their consistent help. We hope that the meeting proves to be what the ASA deserves it to be! Enjoy!!

Robert E. Brannigan, MD
Barry R. Zirkin, PhD

PROGRAM COMMITTEE

Robert E. Brannigan, MD; Hinsdale, IL (Co-Chair)
Barry R. Zirkin, PhD; Baltimore, MD (Co-Chair)

Annual Meeting - Clinical

Arthur L. Burnett, II, MD; Baltimore, MD
Marc Goldstein, MD; New York, NY
Robert D. Oates, MD; Boston, MA
Jay I. Sandlow, MD; Milwaukee, WI
Peter N. Schlegel, MD; New York, NY

Annual Meeting - Basic Science

Gail A. Cornwall, PhD; Lubbock, TX
Ina Dobrinski, DVM, PhD; Calgary, AB Canada
Mary Ann Handel, PhD; Bar Harbor, ME
Haifan Lin, PhD; New Haven, CT
Sally Perreault Darney, PhD; Cary, NC
Bernard Robaire, PhD; Montreal, QC Canada

Special Symposium

Mohit Khera, MD; Houston, TX (Co-Chair)
Allen D. Seftel, MD, FACS; Camden, NJ (Co-Chair)

EMIL STEINBERGER MEMORIAL LECTURE AWARD

Rudolf Jaenisch, MD, is a professor of biology at the Massachusetts Institute of Technology and a member of the Whitehead Institute for Biomedical Research. Dr. Jaenisch's laboratory's expertise is in epigenetics, reprogramming and stem cells. He began is career as a pioneer making transgenic mice, some of which have produced important advances in understanding cancer, neurological and connective tissue disease and developmental abnormaltities. These methods have been used to explore basic questions such as the role of DNA modification, genomic imprinting, X chromosome inactivation, nuclear cloning and, most recently, the nature of stem cells. The laboratory is known for its expertise in cloning mice and in studying the many factors that contribute to the success and failure of that process. They have gained important insights into therapeutic cloning, and have indeed rescued mice having a genetic defect through therapeutic cloning and gene therapy. In addition, using mice as a model and a technique called "altered nuclear transfer," they have demonstrated that it is possible to procure embryonic stem cells without harming a viable embryo. More recently the lab has demonstrated that somatic cells can be reprogrammed *in vitro* to pluripotent ES-like cells and that these cells are suitable to correct both genetic and induced defects in mice by transplantation therapy. Using this technique for turning skin cells into stem cells, the lab has been able to cure mice of sickle cell anemia—the first direct proof that these easily obtained cells can reverse an inherited disease. His group offers a team of scientists who are experts in deriving and manipulating iPS cells, and generating and differentiating neurons from them. Their interest in neurodegenerative diseases such as Parkinson's, Alzheimers, and Synucleinopathy has also created powerful synergies with other laboratories, sharing their expertise to understand and solve these devastating diseases. Dr. Jaenisch has mentored over 32 former predoctoral fellows and over 60 postdoctoral researchers, including current full Professors at Harvard, Stanford and UCLA. Dr. Jaenisch received his medical degree from the University of Munich in Germany and postdoctoral degrees in both molecular/cell biology and developmental biology from Max-Planck-Institute for Biochemistry in Munich and Princeton University in Princeton, New Jersey, respectively. Dr. Jaenisch has received many honors throughout his career, most recently he received both the Franklin Institute Laureate and the Passano Foundation Award in2013. He is on the Editorial Board for *PNAS*, a fellow of the American Academy of Arts and Science and a member of the Internation Society for Stem Cell Research, the National Scademy of Sciences, the German Academy of Natural Sciences Leopoldina and the National Institute of Medicine.

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
2012 William F. Crowley, Jr.
2013 Deborah O'Brien, PhD

DISTINGUISHED ANDROLOGIST AWARD

Gail S. Prins, PhD, is the Michael Reese Professor in the Departments of Urology and Physiology & Biophysics at the University of Illinois at Chicago, College of Medicine. She obtained her PhD in Physiology from the University of Illinois Medical Center, Chicago in 1979 under the tutelage of Laurens Zaneveld, PhD, DVM, a founding member of ASA, with focused studies on sperm transport mechanisms in the vas deferens. She next completed an NIH postdoctoral fellowship in the Department of Urology at Northwestern University Medical School where she developed a research focus on hormonal regulation of the prostate gland. In 1983, Dr. Prins joined the faculty at Michael Reese Hospital & Medical Center as assistant professor of obstetrics and gynecology, University of Chicago. As the founder and director of the In-Vitro Fertilization Laboratory, she was successful in obtaining the first pregnancies and live births in the Midwest using this new technology. She simultaneously built an active a clinical andrology laboratory and a basic research program in prostate androgen receptor regulation. In 1996, Dr. Prins joined the Department of Urology at the University of Illinois at Chicago, moving her research team and clinical andrology laboratory to the UIC campus where she rose through the ranks to her current position.

Dr. Prins has maintained two active and highly successful research programs since the 1980s. Her translational research on human sperm cryopreservation led to the development of an optimal sperm freezing system widely used throughout the globe for both donor and surgically retrieved patient sperm samples. Her basic research program, continuously funded by the NIH for the past 25+ years, is focused on prostate gland development, steroid receptors, hormonal carcinogenesis, endocrine disrupting chemicals (EDCs) and the fetal basis of adult prostate disease. Her work has established that early life exposures to natural estrogens or EDCs, such as bisphenol A, permanently reprogram the prostate and increase its susceptibility to cancer with aging. She has gone on to identify the molecular basis for altered prostate memory, which includes epigenetic reprogramming of prostate stem cells. Most recently, her research team developed novel models to examine these interactions in human embryonic stem cells and prostate epithelial stem and progenitor cells and determined that similar to animal models, stem cell reprogramming and carcinogenic susceptibility are modulated by estrogens and EDCs in the human tissue.

Dr. Prins is widely acclaimed for her research and has authored over 160 peer-reviewed manuscripts in addition to book chapters and position papers. Dr. Prins has performed prodigious service for the ASA over the past 30 years, acting as treasurer (1994 – 1998), president (2003 – 2004), member of the Executive Council, chair of the Finance Committee and chair of the Development Committee as well as service on numerous committees and programs. Similarly, she has served on multiple scientific advisory panels including the Integration Panel for the DoD Prostate Cancer Research Program, the NIEHS External Scientific Review Committee, the NAS Committee to evaluate Veterans and Agent Orange, as NIH grant reviewer and as chair of the Gordon Research Conference on Hormones, Development and Cancer. She is currently an editor of *Endocrinology* and associate editor of *Andrology*. Dr. Prins is the recipient of multiple awards including the Distinguished Service Award from the American Society of Andrology (2001), the Ex-

cellence in Urologic Research Award from the Society for Basic Urologic Research, the 2011 UIC Researcher of the Year Award in Basic Life Sciences and was invested with the Michael Reese Endowed Professorship in Urology at UIC in 2013.

In summary, Dr. Prins experiences a robust career that spans multiple areas of the Andrology field, from basic research discoveries that define the developmental basis of adult prostate disease, to service as a Director of Andrology and IVF Laboratories with translational advances for sperm banking and male infertility. Combined with her career-long scientific service contributions, she exemplifies the qualities embodied in the ASA Distinguished Andrologist award. As such, it is most fitting that the ASA honors her outstanding contributions by bestowing on her the Society's highest honor for 2014.

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
2012	Erwin Goldberg
2013	Christina Wang

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

DISTINGUISHED SERVICE AWARD

Dr. Susan Rothmann is the founder and President of Fertility Solutions Inc. She received a BA in biology from Wells College in 1971, followed by an MS in 1973 and a PhD in 1976 from New York University. She completed postdoctoral fellowship training in Cardiovascular Research and Laboratory Hematology at the Cleveland Clinic Foundation and was a member of the Professional Staff from 1978 to 1992. She founded the Cleveland Clinic Sperm Bank and Andrology Laboratory. She received a Certificate in Health Care Practice Management in 1991 from the Weatherhead School of Management at Case Western Reserve University. Dr. Rothmann holds Board-certifications as High-Complexity Laboratory Director, Andrology Laboratory Director and Clinical Laboratory Consultant. She has post-graduate training in hypnotherapy, guided imagery and business management.

Dr. Rothmann's research interests focus on standardization of sperm morphology classification, education in semen analysis and improved standardization of semen analysis in multicenter clinical trials for vasectomy, pharmaceutical safety and toxicology. Her recent NIH sponsored research identified significant lack of consensus in application of morphology systems, from which she developed a novel standardized method for sperm shape classification based on a dichotomous tree algorithm. In 1992, Dr. Rothmann started work on new methods for teaching laboratory andrology methods and management. She introduced a new format for ASA workshops using interactive exercises and small breakout group modules. She has served as faculty in the majority of them and program chair or co-chair of four, including the first hands-on wet workshop sponsored by the ASA outside of the Annual Meeting. She is currently editing interactive training texts on semen analysis and sperm morphology and developing medical technology curriculum for semen analysis.

Dr. Rothman has authored over 100 scientific manuscripts, book chapters and abstracts, been the recipient of numerous research grants and trained many fellows and students. She is the author/editor of four books on semen analysis and the author/narrator of four fertility guided imagery audios.

Dr. Rothmann joined ASA in 1985 and is a Life Member. She was elected to Executive Council in 1991 and again in 2010. She has chaired the Andrology Laboratories, Endowment Ad Hoc and Awards Committees and is currently chair of Endowment and Development Committee and the 2013 – 2014 Annual Fund Campaign. She served on Student Affairs, Industrial Relations, Nominating, Student Affairs, Liaison, Constitution/Bylaws, Local Arrangements, Laboratory Scientists and Nominating, Endowment and Development and Strategic Planning Committees. She was active in the CASA User Group and represented ASA interests in the College of American Pathologists' Reproductive Biology Resource Committee. She was a founder of Women in Andrology to promote diversity in society leadership. Dr. Rothmann coauthored the 2012 ASA Strategic Plan and wrote the 2013 comprehensive Endowment Plan.

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
2012	Terry R. Brown
2013	Rex A. Hess

YOUNG ANDROLOGIST AWARD

Dr. Sarah Kimmins received her PhD from Dalhousie University in 2003 and completed her postdoctoral training at the Institut de génétique et de biologie moleculaire et cellulaire of the Université Louis Pasteur in Strasbourg, France. She was appointed to the Department of Animal Science in the Faculty of Agricultural and Environmental Sciences in 2005 and is a tenured associate professor. She is an associate member of the Department of Pharmacology and Therapeutics at McGill. She holds a Tier II Canada Research Chair in Epigenetics, Reproduction and Development. Her independent and collaborative research programs have received peer reviewed funding from the Canadian Institutes of Health Research (CIHR), Genome Quebec, Fonds québéc de la recherché sur la nature et les technologies (FQRNT) and the National Sciences and Engineering Research Council (NSERC).

Globally the prevalence of diabetes, obesity and other chronic diseases such as cancer, and cardiovascular disease are on the rise. These increases have occurred at rates that cannot be due to changes in the genetic structure of the population and are likely caused by environmental factors that modify gene function via epigenetics. Kimmins leads a research program in determining how the environment (drugs, nutrients and toxicants) impacts the health of parents and offspring, with a focus on understanding the epigenome in development and disease**.** The epigeome is heritable layer of information that functions like a 'switch' to turn genes on or off. It is transmitted from one generation to the next in the gametes (sperm and egg). Her research involves long-term multi-generational studies to identify the mechanisms implicated in epigenetic inheritance. With her collaborators, she uses transgenic and environmental exposures in rodent models and human samples, in combination with next generation highthroughput technologies to identify the epigenetic signatures that can be transferred from one generation to the next. This is an emerging research area and to date there are only a handful of groups engaged in this kind of research. In 2013 her research group was the first to identify that a father's diet has the ability to
alter development of the embryo and highlights the importance of recognition that the father's preconception health may be equally as important as the mother in terms of having healthy babies. In
particular this research identified folate as a factor in male pre-conception health and its deficiency was associated with increased birth defects in offspring. This ongoing line of research has the potential to impact child health worldwide in terms of prevention of birth defects and chronic disease. This research was highlighted in international Media such as the Washington Post, the LA Times, The Guardian, Time, The Economist, The Globe and Mail, BBC UK, CBC and Global News. This line of research is on the verge of being translated into human studies, pending funding, for long-term studies to follow parents and their offspring in relation to environmental components such as diet and obesity.

Her expertise in epigenomics, development and reproduction is often sought and she serves as a peer reviewer for nation and international granting agencies and for general interest high impact journals as well as field specific journals. Kimmins is an active collaborator with researchers within McGill, Quebec and Internationally. She is extensively involved and a committed member of several international societies and serves on multiple society committees.

YOUNG ANDROLOGIST AWARD RECIPIENTS

1982	L.J.D. Zaneveld
1983	William B. Neaves
1984	Lonnie D. Russell
1985	Bruce D. Schanbacher
1986	Stephen J. Winters
1987	Ilpo T. Huhtaniemi
1988	Larry Johnson
1989	Barry T. Hinton
1990	Luis Rodriguez/Rigau
1991	Patricia M. Saling
1992	Gary R. Klinefelter
1993	Robert Chapin
1994	Wayne J.G. Hellstrom
1995	Christopher DeJonge
1996	Paul S. Cooke
1997	Gail A. Cornwall
1998	William R. Kelce
1999	Stuart E. Ravnik
2000	Matthew P. Hardy
2001	Jacquetta Trasler
2002	Christopher L.R. Barratt
2003	Joanna E. Ellington
2004	Kate Loveland
2005	Janice Bailey
2006	Janice P. Evans
2007	John K. Amory
2008	Moira K. O'Bryan
2009	Michael A. Palladino
2010	Peter Liu
2011	Humphrey Yao
2012	Wei Yang
2013	Jacques J. Tremblay

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

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 2014 Outstanding Trainee Investigator Award will be announced during the Annual Business Meeting on Monday, April 7, 2014 at 5:00 p.m.

NEW INVESTIGATOR AWARD RECIPIENTS

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

OUTSTANDING TRAINEE INVESTIGATOR AWARD RECIPIENTS

2004	Darius Paduch
2005	Tara Barton
2006	Liwei Huang
2007	Steve Tardif
2008	Duangporn Jamsai
2009	Catherine Itman
2010	Michael Elliott
2011	Matthew Marcello
2012	Andrew Major
2013	Mary Samplaski

THANK YOU TO DONORS & SPONSORS

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

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Bayard T. Storey, PhD
Donna L. Vogel, MD, PhD
Christina Wang, MD

Silver Level
(Multiple or single contribution(s) greater than or equal to $5,000)
Erwin Goldberg, PhD
Rex A. Hess, MS, PhD
Ronald W. Lewis, MD, FACS
Gail S. Prins, PhD
J. Lisa Tenover, MD, PhD

Sustaining
(Multiple or single contribution(s) greater than or equal to $2,000)
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William J. Bremner, MD, PhD
Richard Van Clark, MD, PhD
Glenn R. Cunningham, MD
E. Mitch Eddy, PhD
Frank S. French, MD
Wayne J.G. Hellstrom, MD
Joel L. Marmar, MD
Jon Lee Pryor, MD
Bernard Robaire, PhD
Barbara M. Sanborn, PhD
Richard J. Sherins, MD
Terry T. Turner, PhD

Annual Contributions for 2012 (through 11/29/12)

$1000+
Andrzej Bartke, PhD
Douglas T. Carrell, PhD
Anna Steinberger, PhD
Christina Wang, MD

$250 – $999
Richard Van Clark, MD, PhD
Rex A. Hess, MS, PhD
Barry T. Hinton, PhD
J. Lisa Tenover, MD, PhD
Donna L. Vogel, MD, PhD

$100 – $249
F. Kent Hamra, PhD
Wylie C. Hembree, MD
Carin V. Hopps, MD
Bahnam Kazemi, MD
Michael A. Palladino, PhD
Bayard T. Storey, PhD
Jan Karol Wolski, MD

$50 – $99
Arthur T. Burnett, II, MD
Janice P. Evans, PhD
George L. Gerton, PhD
Patricia A. Martin-DeLeon, PhD
Sandra Maria Miraglia
Patricia L. Morris, PhD
Hitoshi Takeshima, MD

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EDUCATIONAL NEEDS & OBJECTIVES

39th Annual ASA Meeting

"*Andrology Where Are We and Where Are We Going?*"

Needs

Male fertility and sexual health are central to men's health in general. Increasingly, new tools, approaches and therapies are becoming available with which to deal with the regulation of fertility and the improvement of health. The use of these modern approaches requires the integration of physiology, endocrinology, genetics, neurobiology and psychology, along with consideration of lifestyle and environmental exposures. There must be extensive interactions among clinicians and translational scientists in order to both recognize and treat clinical conditions related to male fertility and reproductive health.

The 39th Annual Meeting of American Society of Andrology will provide a forum for clinicians and basic scientists to exchange ideas and raise new clinically applicable questions that can lead to novel research directions and efficacious therapies. Renowned researchers working in the fields of urology, endocrinology, clinical andrology, genetics, reproductive medicine and reproductive biology will come together to present cutting edge developments in the physiological and molecular foundations of male reproductive function.

Educational Objectives

- Explain approaches to derive and use induced pluripotent stem cells both for understanding and treatment of a number of diseases of the male reproductive system.
- Describe the standards used for vasectomy and vasectomy reversal, and discuss the controversies that exist.
- Identify the mechanisms that regulate the formation of the stem cell pool in the testis from which spermatozoa ultimately are derived, and the stem cell-based techniques that have potential to generate or regenerate spermatogenesis and thus restore fertility.
- Describe the biochemical pathways that are altered with the occurrence of mutations in the testis that confer advantages to the cells that acquire them, how these pathways provide selective advantages that result in unexpectedly high incidence of the mutations and why particular mutations almost always originate in the father.
- Describe the identification and characterization of germ cell-specific genes that are required for spermatogenesis, and how the protein products of these genes might represent novel, druggable targets for contraception.
- Describe studies designed to determine whether the human prostate is sensitive to the environmental toxicant, bisphenol A, as is the case of the rodent prostate.
- Identify the criteria used by healthcare providers to provide testosterone replacement therapy to older male patients, including the potential benefits and risks.
- Describe how sperm maturation is regulated in the epididymis, and how the sperm become competent for fertilization in the female tract.
- Explain how testosterone production is regulated in the testis and brain, and what the long-term and short-term adverse effects are of altered testicular and neurosteroid levels.
- Identify the evidence for PSA screening in relationship to prostate cancer detection, including the arguments for and against a targeted screening approach.
- Describe pharmacogenetic approaches for the use of follicle-stimulating hormone in the treatment of hypogonadotropic hypogonadism and infertility.
- Describe the mechanistic relationships among environmental exposures and health risk, and the development of cost-effective approaches for efficiently prioritizing the toxicity testing of chemicals.

ASA SPECIAL SYMPOSIUM

Needs

Last year testosterone was one of the fastest growing medications in the United States. However, there continues to be several areas of controversy associated with this medication. Historically there has

been data to support that testosterone may be protective for cardiovascular disease. In fact, many studies have demonstrated that those men with low testosterone levels are much more likely to die from a cardiovascular event. However, recent data suggest that testosterone may be dangerous for cardiovascular health. The FDA has also recently stated that they will be investigating the risk of testosterone on cardiovascular health.

Today the main reason why clinicians do not prescribe testosterone is the fear that it may cause prostate cancer. However, there is no compelling data to support this. Many clinicians are unaware of the published data on testosterone and prostate cancer.

Finally many clinicians are still unaware that giving testosterone can reduce sperm counts. A recent AUA survey demonstrated at most Urologist would give men who are trying to conceive testosterone. Testosterone acts as a natural contraceptive and many clinicians still need to be educated on the impact of testosterone on fertility.

Educational Objectives
By the end of the ASA Symposium, attendees should be able to:

- Explain the controversy of testosterone and cardiovascular disease.
- Describe how testosterone affects the heart and overall cardiac function.
- Describe how to treat hypogonadal men who wish to preserve their fertility.
- Identify the cardiovascular and metabolic risks associated with low testosterone and how low testosterone is associated with an increased risk of mortality.
- Describe the published literature on testosterone and prostate cancer.
- Describe the effects of testosterone on the prostate and how testosterone can be used to treat men following radical prostatectomy.
- Explain how to recover sperm function in those men who have abused testosterone.

ACCREDITATION INFORMATION

Accreditation Statement
This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of Oklahoma College of Medicine and the American Society of Andrology. The University of Oklahoma College of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

The University of Oklahoma College of Medicine designates this live activity for a maximum of 21.00 *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Conflict Resolution Statement
The University of Oklahoma College of Medicine, Office of Continuing Professional Development has reviewed this activity's speaker and planner disclosures and resolved all identified conflicts of interest, if applicable.

Equal Opportunity Statement
The University of Oklahoma is an equal opportunity institution. www.ou.edu/eoo

Accomodation Statement
Accommodations on the basis of disability are available by contacting (847) 619-4909 by as soon as possible.

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Appropriate acknowledgement will be given to all supporters at the time of the educational activity.

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It is the policy of the University of Oklahoma College of Medicine that the faculty and sponsor disclose real or apparent conflicts of interest relating to the topics of this educational activity, and also disclose discussions of unlabeled/unapproved uses of drugs or devices during their presentation(s). Detailed disclosures will be made in the course handout materials.

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MARK YOUR CALENDARS

ASA 40th Annual Conference
April 18 – 21, 2015
Little America Hotel
Salt Lake City, UT

Andrology Lab Workshop
April 18 – 19, 2015

Testis Workshop "Healthy Sperm/Healthy Children"
April 15 - 18, 2015

ASA Special Symposium
April 18, 2015

SCHEDULE OF EVENTS

ASA 39th Annual Conference
"Andrology: Where Are We and Where Are We Going?"
April 5 – 8, 2014
InterContinental Buckhead Atlanta
Atlanta, Georgia

Program Chairs: Robert E. Brannigan, MD and Barry R. Zirkin, PhD

Location: Windsor Ballroom C-E

FRIDAY, APRIL 4, 2014

2:00 p.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

SATURDAY, APRIL 5, 2014

7:30 a.m. – 7:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

4:00 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

8:30 a.m. - 4:00 p.m. **ASA Basic Science Workshop**
(See pg. 27 for full program)

9:00 a.m. - 5:00 p.m. **ASA Andrology Workshop**
(See pg. 28 for full program)

1:00 p.m. - 5:15 p.m. **ASA Special Symposium**
(See pg. 29 for full program)

5:30 p.m. – 5:40 p.m. **Welcome and Opening Remarks**

5:40 p.m. – 6:00 p.m. **Updates from NICHD & NIEHS: Where Are We and Where Are We Going?**
Stuart B. Moss, PhD
NICHD
Thaddeus T. Schug, PhD
NIEHS

6:00 p.m. – 6:20 p.m. **Distinguished Andrologist Award**

6:20 p.m. – 6:30 p.m. **Centers for Disease Control and Prevention Welcomes ASA to Atlanta**
Hubert Vesper, PhD
National Center for Environmental Health

6:30 p.m. – 7:30 p.m. **EMIL STEINBERGER MEMORIAL LECTURE**
iPS Cell Technology and Disease Research: Issues to be Resolved
Rudolf Jaenisch, MD
Massachusetts Institute of Technology
(Introduced by Erwin Goldberg, PhD)

7:30 p.m. – 9:30 p.m. **Welcome Reception**
Location: Windsor Ballroom AB

SUNDAY, APRIL 6, 2014

6:30 a.m. – 8:00 a.m. **Past President's Breakfast**
Location: Trippe 1

6:30 a.m. – 6:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

7:00 a.m. – 4:00 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

7:00 a.m. – 8:00 a.m. **Continental Breakfast in Exhibit Hall**
Location: Windsor Ballroom AB

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
Controversies in Vasectomy and Vasectomy Reversal
Jay I. Sandlow, MD
Medical College of Wisconsin
(Introduced by Robert E. Brannigan, MD)

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

9:15 a.m. – 10:45 a.m. **SYMPOSIUM I – Stem Cells in the Male Reproductive Tract**
Co-chairs: Marie-Claude Hofmann, PhD; Makoto Nagano, PhD, DVM

Unraveling Signaling Pathways Controlling Gonocyte Differentiation
Martine Culty, PhD
McGill University

SCHEDULE OF EVENTS

Regulation of Spermatogonial Stem Cells in the Adult Testis
William Wright, PhD
Johns Hopkins Bloomberg School of Public Health

Human and Non-Human Primate Stem Cells
Kyle Orwig, PhD
University of Pittsburgh School of Medicine

10:45 a.m. – 11:00 a.m. **Break**
Location: Windsor Ballroom AB

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

12:30 p.m. – 2:00 p.m. **MENTORING LUNCHEON SPONSORED BY THE DIVERSITY AND TRAINEE AFFAIRS COMMITTEES**
Embarking on a Scientific Career: Combining Administrative, Teaching and Clinical Responsibilities
Location: Trippe 1
William J. Bremner, MD, PhD
University of Washington
(Introduced by Peter Liu, MBBS, PhD)
**Not included in registration; ticket required*

12:30 p.m. – 2:00 p.m. **Editorial Board Luncheon**

12:30 p.m. – 2:00 p.m. **Lunch On Your Own**

CONCURRENT ORAL SESSIONS

2:00 p.m. – 3:30 p.m. **Oral Session I: Molecular and Environmental Regulation of Male Reproductive Health**
Location: Windsor C - E
Moderators: Kate Loveland, PhD; Jacquetta M. Trasler, MD, PhD

2:00 p.m. – Abstract #1
RESPONSIVENESS OF THE SPERMATOGONIAL STEM CELL POOL TO RETINOIC ACID
Ryan Anderson, BS, Melissa Oatley, MS and Jon Oatley, PhD
Washington State University
(Presented By: Jon Oatley, PhD)

2:15 p.m. – Abstract #2
THE TRANSLATIONAL REPRESSOR, Y-BOX PROTEIN 2 (YBX2/MSY2), BINDS THE CIS-ELEMENT (TCE) THAT INACTIVATES MOUSE PRM1 MRNA TRANSLATION IN ROUND SPERMATIDS.
Tamjid Chowdhury, BA and Kenneth Kleene, PhD
University of Massachusetts Boston
(Presented By: Kenneth Kleene, PhD)

2:30 p.m. – Abstract #3
KDM1A OVEREXPRESSION IN MOUSE TESTES ALTERS THE EPIGENETIC LANDSCAPE OF SPERM HISTONES AND IS IMPLICATED IN TRANSGENERATIONAL INHERITANCE
Keith Siklenka, Serap Erkek[1], Maren Godmann[2], Romain Lambrot[2], Christine Lafleur[2], George Chountalos[2], Tamara Cohen[2], Marilene Paquet[2], Matthew Suderman[2], Mike Hallett[2], Serge McGraw[2], Donovan Chan[2], Jacquetta Trasler[2], Antoine Peters[1] and Sarah Kimmins[2]
[1]Friedrich Miescher Institute for Biomedical Research, Switzerland; [2]McGill University, Canada
(Presented By: Keith Siklenka)

2:45 p.m. – Abstract #4
CHRONIC EXPOSURE TO LOW DOSES OF DI-N-BUTYL PHTHALATE (DBP) RESULTS IN SMALLER TESTES, ABNORMAL TESTOSTERONE LEVELS, IMPAIRED BONE HEALTH AND GREATER WEIGHT GAIN IN ADULT MICE.
Sarah Moody, BBiomedSci[1], Hoey Goh, BBiomedSci[1], Rachelle Johnson, PhD[2], Natalie Sims, PhD[2], Kate Loveland, PhD[1] and Catherine Itman, PhD[3]
[1]Monash University; [2]St. Vincent's Institute; [3]University of Newcastle
(Presented By: Catherine Itman, PhD)

3:00 p.m. – Abstract #5
PRENATAL EXPOSURE TO AN ENVIRONMENTALLY-RELEVANT CONTAMINANT MIXTURE ALTERS THE EPIGENOME OF FATHERS, DECREASES THEIR FERTILITY AND THE HEALTH OF THEIR SONS IN A RAT MODEL.
Clotilde Maurice, PhD Student[1], Serge McGraw, PhD[2], Arnaud Droit, PhD[1], Jacquetta Trasler, MD, PhD[2], Sarah Kimmins, PhD[2] and Janice Bailey, PhD[1]
[1]Université Laval; [2]McGill University
(Presented By: Clotilde Maurice, PhD Student)

3:15 p.m. – Abstract #6
PRENATAL EXPOSURE TO A COMBINATION OF ENDOCRINE DISRUPTORS EXACERBATES EARLY AND LONG TERM EFFECTS ON MALE REPRODUCTIVE HEALTH AND DEVELOPMENT
Steven Jones, MSc[1], Annie Boisvert, MSc[2], Peter Thrane, BSc[3], Sade Francois, BSc[4] and Martine Culty, PhD[5]
[1]McGill University, Research Institute of the MUHC, Division of Experimental Medicine, Montreal, Quebec; [2]McGill University, Research Institute of the MUHC and Department of Medicine; [3]McGill University, Research Institute of the MUHC; [4]McGill University, Research Institute of the MUHC and Department of Pharmacology and Therapeutics; [5]McGill University, Research Institute of the MUHC, Division of Experimental Medicine and Departments of Medicine and Pharmacology and Therapeutics
(Presented By: Steven Jones, MSc)

SCHEDULE OF EVENTS

2:00 p.m. – 3:30 p.m. **Oral Session II: Human Spermatogenesis: Novel Findings in 2014**

Location: Hope

Moderators: Dolores J. Lamb, PhD
Kyle Orwig, PhD

2:00 p.m. – Abstract #7
LEVELS OF THE RETINOIC ACID SYNTHESIZING ENZYME ALDH1A2 ARE LOWER IN TESTICULAR TISSUE FROM MEN WITH INFERTILITY
John Amory, MD, MPH[1], Samuel Arnold, MS[1], Maria Lardone, MS[2], Antonio Piottante, MD[3], Mauricio Ebensperger, MD[4], Nina Isoherranen, PhD[1], Charles Muller, PhD[1], Thomas Walsh, MD[1] and Andrea Castro, MS[2]
[1]University of Washington; [2]University of Chile; [3]Andres Bello University; [4]San Borja Arriaran Hospital
(Presented By: John Amory, MD, MPH)

2:15 p.m. – Abstract #8
A MICROARRY ANALYSIS OF UNIQUE GENES FOUND IN MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA (NOA) AND VARICOCELES.
Jason Kovac, MD, PhD, Josephine Addai, BSc, Larry Lipshultz, MD and Dolores Lamb, PhD
Baylor College of Medicine
(Presented By: Jason Kovac, MD, PhD)

2:30 p.m. – Abstract #9
MICRORNA EXPRESSION IN MEN WITH CONFIRMED DIAGNOSIS OF EARLY MATURATION ARREST
Ali Dabaja, MD, Anna Mielnik, MS, Matthew S. Wosnitzer, MD, Peter N. Schlegel, MD and Darius A. Paduch, MD, PhD
Weill Cornell Medical College
(Presented By: Ali Dabaja, MD)

2:45 p.m. – Abstract #10
MALE INFERTILITY FROM OVERUSE OF MEDICAL TESTOSTERONE IN MEN IN THIER REPRODUCTIVE YEARS – AN UNNECESSARY PROBLEM
William Parker, MD, Brian McArdle, DO, Arash Sattarin, Zachary Hamilton, MD and Ajay Nangia, MD
The University of Kansas
(Presented By: William Parker, MD)

3:00 p.m. – Abstract #11
POST-FINASTERIDE PERSISTENT SIDE EFFECTS MAY BE ASSOCIATED WITH PERSISTENT 5 ALPHA-REDUCTASE INHIBITION: A PILOT STUDY
Seth Cohen, MD, MPH
(Presented By: Seth Cohen, MD, MPH)

3:15 p.m. – Abstract #12
RECOVERY OF UNDIFFERENTIATED SPERMATOGONIA FROM THE TESTES OF PREPUBERTAL PATIENTS AFTER EXPOSURE TO CHEMOTHERAPY
Hanna Valli[1], Karen A. Peters[2], Brian P. Hermann[3], Meena Sukhwani[2], Peter H. Shaw[4], Joseph S. Sanfilippo[5], Thomas M. Jaffe[6] and Kyle E. Orwig[7]
[1]Departments of Obstetrics, Gynecology & Reproductive Sciences, Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine; [2]Magee-Womens Research Institute, Pittsburgh, PA 15213; [3]Department of Obstetrics, Gynecology & Reproductive Sciences and Magee-Womens Research Institute, Pittsburgh; [4]Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260; [5]Departments of Obstetrics, Gynecology & Reproductive Sciences, Center for Fertility and Reproductive Endocrinology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260; [6]Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260; [7]Departments of Obstetrics, Gynecology & Reproductive Sciences, Center for Fertility and Reproductive Endocrinology, Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260. Magee-Womens Research Institute, Pittsburgh, PA 15213
(Presented By: Hanna Valli)

3:30 p.m. – 4:00 p.m. **Break**
Location: Windsor AB

4:00 p.m. – 4:45 p.m. **LECTURE I**
What's Good for the Spermatogonial Stem Cell May Be Bad for the Offspring: Advantageous Mutations that Increase the Incidence of Human Disease
Norman Arnheim, PhD
University of Southern California
(Introduced by Mary A. Handel, PhD)

4:45 p.m. – 5:30 p.m. **LECTURE II**
Novel Spermatogenic Pathways and Male Contraception
Martin M. Matzuk, MD, PhD
Baylor College of Medicine
(*Introduced by Jacquetta M Trasler, MD, PhD*)

5:30 p.m. – 6:15 p.m. **SYMPOSIUM – Updates from the Centers for Disease Control and Prevention: Progress in Male Reproductive Health**
Moderator: Steven M. Schrader, PhD
National Institute for Occupational Safety and Health, CDC

SCHEDULE OF EVENTS

Insights Gained from CDC Surveys and Initiatives
Lee Warner, PhD
National Center for Chronic Disease Prevention and Health Promotion, CDC

CDC's Hormone Standardization Program: A Focus on Testosterone
Hubert Vesper, PhD
National Center for Environment Health, CDC

Discussion: Potential Collaboration with Academic Programs and National Organizations

6:30 p.m. – 8:30 p.m. **Trainee Forum and Mixer**
Location: Venetian
**All Trainee Travel Awards will be distributed and celebrated at this event*

MONDAY, APRIL 7, 2014

7:00 a.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

7:00 a.m. – 8:00 a.m. **Continental Breakfast**
Location: Windsor Foyer

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Hormone Signaling and Reprogramming in Human Prostate Stem Cells
Gail S. Prins, PhD
University of Illinois at Chicago
(*Introduced by Donna L. Vogel, MD, PhD)*

9:00 a.m. - 9:15 a.m. **Young Adrologist Award**

9:15 a.m. – 10:15 a.m. **SYMPOSIUM II – Would You Give This Man Testosterone? Case-Based Discussions**
Moderators: Christina Wang, MD
Stephanie T. Page, MD, PhD

J. Lisa Tenover, MD, PhD
VA Palo Alto Health Care System
Peter N. Schlegel, MD
The New York Weill/Cornell Medical Ctr.

10:15 a.m. – 10:30 a.m. **Break**
Location: Windsor Foyer

10:30 a.m. – 11:15 a.m. **DIVERSITY LECTURE**
Disparities in Men's Health: The Role of the Primary Care Physician
Charles S. Modlin, MD
Cleveland Clinic Foundation, Minority Men's Health Center
(Introduced by George L. Gerton, PhD)

11:15 a.m. – 12:30 p.m. **Poster Session II**
Location: Venetian

12:30 p.m. – 1:45 p.m. **WOMEN IN ANDROLOGY LUNCHEON AND DISCUSSION**
What Successful Women Do Differently: Learning To Embrace Failure and To Take Risks
Location: Trippe
Moderator: Sophie La Salle, PhD
**Not included in registration fee; ticket required*

1:45 p.m. – 3:15 p.m. **SYMPOSIUM III Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility**
Co-Chairs: Gail A. Cornwall, PhD
Kenneth P. Roberts, PhD

Qualitative and Quantitative Aspects of the Hormonal Control of Spermatogenesis Revisited
Ilpo Huhtaniemi, MD, PhD, FMedSci
Imperial College, London

Ca2+ and cAMP Signaling Crosstalk During Sperm Capacitation
Pablo E. Visconti, PhD
University of Massachusetts

Aging Affects Germ Cells: From Genes to Fertility
Bernard Robaire, PhD
McGill University

3:15 p.m. – 3:30 p.m. **Break**
Location: Windsor Foyer

SCHEDULE OF EVENTS

3:30 p.m. – 4:15 p.m. **LECTURE III:**
The Stress Hormone Corticotropin-Releasing Factor Acts in the Brain and the Testes to Regulate Testosterone Secretion
Catherine Rivier, PhD
The Salk Institute for Biological Studies
(Introduced by Vassilios Papadopoulos, PhD)

4:15 p.m. – 5:00 p.m. **LECTURE IV:**
Pharmacological Regulation of Steroid Biosynthesis: From Testis to Brain
Vassilios Papadopoulos, PhD
The Research Institute of the McGill
University Health Centre
(Introduced by Catherine Rivier, PhD)

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

7:30 p.m. – 11:00 p.m. **Annual Banquet**
Location: Atlanta Event Center at Opera
Buses will be leaving from hotel lobby at 6:45 p.m.
**Not included in registration fee; ticket requried*

TUESDAY, APRIL 8, 2014

7:00 a.m. – 8:00 a.m. **2015 Program Committee Meeting**
Location: Hope

7:30 a.m. – 12:15 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

7:00 a.m. – 8:00 a.m. **Continental Breakfast**
Location: Windsor Foyer

8:00 a.m. – 9:15 a.m. **SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?**
Moderators: Gail S. Prins, PhD
Arthur L. Burnett, II, MD
William J. Catalona, MD
Northwestern University
Timothy Wilt, MD
University of Minnesota School of Medicine
Herbert B. Carter, MD
Johns Hopkins Hospital

9:15 a.m. – 10:15 a.m. **INTERNATIONAL LECTURE:**
Pharmacogenetics of FSH
Manuela Simoni, MD, PhD
University of Modena and Reggio Emilia, Italy
(Introduced by Patricia S. Cuasnicu, PhD)

10:15 a.m. – 10:30 a.m. **Break**
Location: Windsor Foyer

10:30 a.m. – 12:00 p.m. **SYMPOSIUM V – Innovations in Male Environmental Health Protection**
Co-Chairs: Sally Perreault Darney, PhD
Bernard Robaire, PhD

Revolution in Toxicity Testing and Risk Prediction for Chemicals in the Environment
Thomas Knudsen, PhD
US Environmental Protection Agency

Response of Human Fetal Testis Xenotransplants to Environmental Toxicants: Implications for Risk Assessment
Kim Boekelheide, MD, PhD
Brown University

Translation of the Science in Male Reproductive and Environmental Health for Evidence-Based Decisions by Clinicians, Regulators and the Public
Paula I. Johnson, PhD
University of California, San Francisco

MEETING ADJOURNED

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Statements, opinions and results of studies contained in the program are those of the presenters/authors and do not reflect the policy or position of the ASA nor does the ASA provide any warranty as to their accuracy or reliability.

SCHEDULE OF EVENTS

***Basic Science Workshop**
"Assessing Male Reproductive Function in the Laboratory"
8:30 a.m. - 4:00 p.m.
April 5, 2014
InterContinental Buckhead Atlanta
Atlanta, Georgia

Chair: Sophie La Salle, PhD

**Not CME Accredited*

*All sessions will be held in **Hope** unless otherwise noted.*

SATURDAY, APRIL 5, 2014

7:30 a.m. – 7:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

4:00 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

8:30 a.m. **Session 1: Visualizing Male Reproductive Capacity**
Rex Hess, PhD, University of Illinois, USA
Dolores Lamb, PhD, Baylor College of Medicine, USA
Gunapala Shetty, PhD, MD Anderson Cancer Center, USA

Break

Session 2: Markers and Mechanisms of Reproductive Function
Development and Assessment of Epididymal Function
Barry Hinton, PhD, University of Virginia USA

Oxidative Stress Markers and Role of Reactive Oxygen Species in Sperm Function
Cristian O'Flaherty, DVM, PhD, McGill University, Canada

Identification and Functional Assessment of Human Spermatogonial Stem Cells
Hooman Sadri-Ardekani, MD, PhD

Epididymal Sperm Preparation from Mice: Key Steps and Challenges
Genevieve Plante, PhD Candidate, Universite de Montreal, Canada

Lunch

Session 3: New Tools and Approaches for Discovery
Visualization of Immunolocalized Proteins in Sperm Using Electron Microscopy
James Foster, PhD, Randolph-Macon College, USA

Application of Patch-Clamping to the Study of Testicular Cell Function
David Fleck, PhD Candidate, RWTH-Aachen University, Germany

Experimental Considerations and Analysis of Testis RNA-Sequence Datasets
Elizabeth Snyder, PhD, The Jackson Laboratory, USA

Interactome Analysis of Spermatogenesis: A Systems Biology Approach to Andrology
Baruk Ozkosem, PhD Candidate, McGill University, Canada

Proteomic Analysis of Proteins Involved in Sperm Capacitation
Ana Maria Saliconi, PhD, University of Massachusetts, USA

Protemic and Biochemical Analysis of Exosomes in Semen
Alan Diekman, PhD, University of Arkansas for Medical Sciences, USA

Simulation of the Mouse Spermatogenic Cycle Using Computer Modeling
Ping Ye, PhD, Washington State University, US

4:00 p.m. Adjourn

SCHEDULE OF EVENTS

***Andrology Lab Workshop**

"Post Vasectomy Semen Analysis: Lab Methods and Interpretation"

April 5, 2014

InterContinental Buckhead Atlanta

Atlanta, Georgia

Program Chairs: Charles H. Muller, PhD, HCLD

**Not CME Accredited*

Location: Tripe

SATURDAY, APRIL 5, 2014

7:30 a.m. – 7:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

4:00 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

9:00 a.m. – 9:15 a.m. **Introduction**
Charles H. Muller, PhD, HCLD

9:15 a.m. – 10:15 a.m. **Methods of Assessing Post-Vasectomy Semen**
Charles H. Muller, PhD, HCLD
Susan Rothman, PhD, HCLD

10:15 a.m. – 10:35 a.m. **Break**

10:35 a.m. – 11:35 a.m. **Clinical Assessment of Post-Vasectomy Semen Analysis and Statistics of Small Numbers**
Charles H.Muller, PhD, HCLD
Susan Rothman, PhD, HCLD

11:35 a.m. – 12:00 p.m. **Exercise and Discussion (Needle in a Haystack) Committee**
ALW Committee

12:00 p.m. – 1:30 p.m. **Lunch with Laboratory Science Forum**

1:30 p.m. – 2:20 p.m. **Vas Recanalization and Vasectomy Failure**
Charles H. Muller, PhD, HCLD

2:20 p.m. – 2:40 p.m. **Break**

2:40 p.m. – 3:30 p.m. **AUA Recommendation: PVSA Office Microscopy Procedure and CLIA Requirements**
ALW Committee
Susan Rothman, PhD, HCLD

3:30 p.m. – 4:10 p.m. **Panel Discussion of AUA Recommendation**
ALW Committee, Faculty, Guests

4:10 p.m. – 4:40 p.m. **Conclusions, Summary, Questions**
Charles H. Muller, PhD, HCLD

4:40 p.m. – 5:00 p.m. **Course Summary and Evaluation**

SCHEDULE OF EVENTS

ASA Special Symposium

"Controversies in Testosterone Therapy: Cardiovascular Disease/Metabolic Syndrome, Prostate Cancer, and Fertility"

April 5, 2014
InterContinental Buckhead Atlanta
Atlanta, Georgia

Program Chairs: Mohit Khera, MD and Allen D. Seftel, MD, FACS

Location: Windsor C-E

SATURDAY, APRIL 5, 2014

7:30 a.m. – 7:30 p.m. **Registration/Information Desk Open**
Location: Windsor Pre-Function Area

4:00 p.m. – 9:30 p.m. **Exhibit Hall Open**
Location: Windsor Ballroom AB

Controversies in Testosterone Therapy and Prostate Cancer
Moderators: Ethan Grober, MD, MEd, FRCSC; Larry Lipshultz, MD

1:00 p.m. – 1:20 p.m. **Testosterone and Prostate Cancer: Understanding the Risks**
Abraham Morgentaler, MD

1:20 p.m. – 1:40 p.m. **Basic Science Review of Testosterone and Prostate Cancer**
Abdulmaged Traish, PhD

1:40 p.m. – 2:00 p.m. **Testosterone for Penile Rehabilitation following Radical Prostatectomy**
Mohit Khera, MD

2:00 p.m. – 2:15 p.m. **Questions and Answers**

Controversies in Testosterone Therapy and Cardiovascular Disease/Metabolic Syndrome
Moderators: Allen Seftel, MD, FACS

2:15 p.m. – 2:35 p.m. **Testosterone and Metabolic Syndrome**
Glenn Cunningham, MD

2:35 p.m. – 3:15 p.m. **Testosterone and Cardiovascular Disease**
Stephanie Page, MD, PhD

3:15 p.m. – 3:30 p.m. **Should Testosterone be a Standard Annual Screen in Men?**
Tobias Kohler, MD, MPH

3:30 p.m. – 3:45 p.m. **Questions and Answers**

3:45 p.m. – 4:00 p.m. **Break**

Controversies in Testosterone Therapy and Infertility
Moderators: Mark Sigman, MD; Edward Kim, MD

4:00 p.m. – 4:20 p.m. **Preserving Fertility in the Hypogonadal Patient**
Larry Lipshultz, MD

4:20 p.m. – 4:40 p.m. **Anabolic Steroid Abuse and Infertility**
Ajay Nangia, MBBS

4:40 p.m. – 5:00 p.m. **Understanding the Role of Testosterone in Fertility**
Dolores Lamb, PhD

5:00 p.m. – 5:15 p.m. **Questions and Answers**

SPEAKER ABSTRACTS

SATURDAY, APRIL 6, 2014
6:30 p.m. – 7:30 p.m.

EMIL STEINBERGER MEMORIAL LECTURE

iPS Cell Technology, Gene Editing and Disease Research: Issues to be Resolved

Rudolf Jaenisch
Whitehead Institute for Biomedical Research and Department of Biology, MIT, Cambridge, MA 02124, USA

The recent demonstration of in vitro reprogramming using transduction of 4 transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of in vitro reprogramming need to be understood and will be one focus of the talk. A major impediment in realizing the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Methods based on Zn finger or TALEN mediated genome editing have allowed to overcome the inefficiency of homologous recombination in human pluripotent cells. Using this genome editing approaches we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. The most recent advance comes from the use of the CRISPR/Cas9 system to engineer ES cells and mice. This technology allows the simultaneous editing of multiple genes and will facilitate establishing relevant models to study human disease.
We have used this technology to generate isogenic pairs of cells that differ exclusively at a disease causing mutation. The talk will describe the use or isogenic pairs of mutant and control iPS cells to establish in vitro systems for the study of diseases such as Parkinson's and Rett syndrome.

SUNDAY, APRIL 6, 2014
8:00 a.m. – 9:00 a.m.

AUA LECTURE

CURRENT STANDARDS AND CONTROVERSIES REGARDING VASECTOMY AND VASECTOMY REVERSAL

Jay Sandlow, MD
Professor and Vice-Chair, Department of Urology, Medical College of Wisconsin, Milwaukee, Wisconsin

Introduction: Vasectomy is a safe and effective method of permanent contraception. In the United States, it is employed by nearly 11% of all married couples and performed on approximately one-half a million men per year, which is more than any other urologic surgical procedure. However, far fewer vasectomies are performed than female sterilizations by tubal ligation, both in the US and worldwide, despite the fact that vasectomy is less expensive and associated with less morbidity and mortality than tubal ligation. Recently, the American Urological Association (AUA) formed a panel of experts to develop evidence-based guidelines on vasectomy. This has been published and has been met with great acceptance; however, questions still exist regarding several aspects of vasectomy which will be addressed in this talk. Conversely, vasectomy reversal is much less common than vasectomy. Approximately 4-6% of men who have had a vasectomy ultimately request a reversal. Reasons vary, from desiring children with a new partner to a couple's desire for more children together, and rarely, due to perceived changes after vasectomy. The main controversies regarding vasectomy reversal is patient selection and cost-effectiveness compared to in vitro fertilization (IVF). The following presentation will review the standards of both procedures (using evidence-based literature when possible), as well as discuss some of the controversies that exist.
Methods: The recent AUA Guidelines were developed using an evidence-based approach. A systematic review of the literature using the MEDLINE and POPLINE databases with search dates January 1949-August 2011 was conducted to identify peer-reviewed relevant publications. The search identified almost 2,000 titles and abstracts. Application of inclusion/exclusion criteria yielded an evidence base of 275 articles. Only a small subset of these articles is referenced in this summary. A complete list of references and a full explanation of AUA guideline methodology can be found in the unabridged text of Vasectomy: AUA Guideline (2012), which is available online at http://www.auanet.org/content/media/vasectomy.pdf. Although there is not a similar document for vasectomy reversal, a group of experts are currently in the process of assembling a literature search to develop similar Best Practice guidelines.
Results: The AUA Guideline on Vasectomy became available in print and on-line in 2012. The document reviews the entire procedure, from counseling to follow up, including best practice for performing the procedure, complications and future areas for research. Literature regarding vasectomy reversal outcomes has demonstrated an overall high success rate for reversal, dependent upon various factors, including time from vasectomy, surgeon training and experience, and most importantly, female partner factors. Multiple studies have reported on cost-effectiveness in comparison to IVF, with most showing lower costs and similar outcomes for reversals. Other studies have examined the role of vasectomy reversal for post vasectomy pain, with good efficacy in carefully chosen patients.
Conclusion: Vasectomy is a highly efficacious, minimally invasive form of permanent contraception. Evidence-based literature has been used to develop guidelines for vasectomy, addressing many of the controversies using published studies. Vasectomy reversal, although somewhat uncommon, provides couples with a cost-effective method for having children after previous vasectomy. Outcomes are dependent upon several factors which should be addressed on an individual basis.

SUNDAY, APRIL 6, 2014
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Stem Cells in the Male Reproductive Tract

UNRAVELING SIGNALING PATHWAYS CONTROLLING GONOCYTE DIFFERENTIATION

Martine Culty, PhD
The Research Institute of the McGill University Health Centre and the Department of Medicine, McGill University, Montreal, Quebec, Canada

Spermatogenesis depends on the formation of a pool of spermatogonial stem cells shortly after birth, constituting a life-long reservoir of cells that will self-renew or form progenitor cells destined to enter the spermatogenic cycle. Spermatogonia arise from the differentiation of neonatal/transitional gonocytes (pre/pro-spermatogonia). Thus, the establishment of an adequate germline stem cell pool, and subsequently male fertility, rely on the ability of gonocytes to proliferate and undergo differentiation. It is our goal to identify the mechanisms regulating gonocyte differentiation, to understand how spermatogonial stem cells are formed and to gain insights into the origins of testicular cancer and infertility.
Neonatal gonocyte differentiation is a tightly regulated process occurring within two to three days in rat, following gonocyte proliferation and migration to the basement membrane of the seminiferous cords. To identify genes that might play a role in gonocyte differentiation, we compared the gene expression profiles of rat neonatal gonocytes and spermatogonia, reflecting in vivo differentiation. Among genes differentially expressed between gonocytes and spermatogonia and during in

vitro gonocyte differentiation were several related to the ubiquitin proteasome system. Indeed, active proteasomal degradation was required for differentiation. Next, we examined the mechanisms involved in in vitro retinoic acid-induced gonocyte differentiation, and identified two signaling pathways crosstalking with the retinoic acid pathway, src and JAK/STAT. We previously reported that gonocyte proliferation also involves signaling crosstalk between PDGF and estradiol, mediated by Erk1/2 activation. Thus, gonocyte functions appear to require the coordinated activation and interaction of several signaling pathways.
These findings suggest that more than one signal transduction pathways are necessary to maintain a tight control of germ cell function within the very dynamic environment of the developing testis, which is flooded by factors regulating somatic cell proliferation and/or differentiation.
Funding provided by NSERC, CIHR, CSR and RQR grants and awards.

SUNDAY, APRIL 6, 2014
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Stem Cells in the Male Reproductive Tract
REGULATION OF SPERMATOGONIAL STEM CELLS IN THE ADULT TESTIS
William W. Wright, PhD
Division of Reproductive Biology, Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Spermatogonial stem cells (SSCs), a subset of the undifferentiated A single (A_s) spermatogonia, are the foundation of spermatogenesis. SSCs give rise to non-stem A_s spermatogonia, which along with the A paired (A_{pr}) and the A aligned (A_{al}) spermatogonia, constitute the transit-amplifying progenitor spermatogonia. It is know that the Sertoli cell product, glial cell line-derived neurotrophic factor (GDNF), is essential for maintaining numbers of these cells, but it is unknown whether the specific cells that this growth factor affects are A_s, A_{pr} and/or A_{al} spermatogonia, or whether SSCs and progenitor spermatogonia are equally responsive to changes in GDNF signaling. To examine these issues we used a chemical-genetic approach to inhibit GDNF signaling in the adult, and identified cells by their expression of GFR□1 and their connection to one or more cells. Results showed that inhibition of GDNF signaling for two days suppressed replication of A_s, A_{pr} and A_{al} spermatogonia. Furthermore, their replication was stimulated when GDNF was added to cultured seminiferous tubules for two days. We next asked whether *in vivo* GDNF suppressed a late step in spermatogonial differentiation, expression of Kit. Results show that *in vivo,* inhibition of GDNF signaling for three or seven days progressively increased the percentages of A_s, A_{pr} and A_{al} spermatogonia expressing this marker of differentiation. Finally, we report that SSCs are lost more slowly than progenitor spermatogonia when GDNF signaling is inhibited. This suggests that SSCs are less responsive to changes in GDNF signaling than are progenitor spermatogonia.

SUNDAY, APRIL 6, 2014
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Stem Cells in the Male Reproductive Tract
HUMAN AND NON-HUMAN PRIMATE STEM CELLS
Kyle E. Orwig, PhD
Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Spermatogonial stem cells (SSCs) are at the foundation of spermatogenesis and may have application for treating some cases of male infertility. This lecture will review similarities and species-specific differences in the stem cell pool and spermatogenic lineage development between mice, monkeys and humans. These comparisons have implications for the experimental tools that can be used to study SSCs in each species as well as the interpretation of data generated using those tools. SSC transplantation is a valuable bioassay of SSC activity and may be used to regenerate spermatogenesis in infertile men. Our results in a preclinical nonhuman primate model of chemotherapy-induced infertility suggest that SSC transplantation can be used to regenerate spermatogenesis in men. The lecture will conclude with a discussion of current challenges of translating the SSC transplantation technology to the clinic.

Funding by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development grants HD055475, HD008610 and HD061289; Magee-Womens Research Institute and Foundation; the Richard King Mellon Foundation and the United States-Israel Binational Science Foundation.

SUNDAY, APRIL 6, 2014
4:00 p.m. – 4:45 p.m.

LECTURE I
WHAT'S GOOD FOR THE SPERMATOGONIAL STEM CELL MAY BE BAD FOR THE OFFSPRING: ADVANTAGEOUS MUTATIONS THAT INCREASE THE INCIDENCE OF HUMAN DISEASE
Norman Arnheim and Peter Calabrese
Molecular and Computational Biology, University of Southern California, Los Angeles, CA USA

Besides disease mutations already present in families, new mutations occur in the germline each generation and may be inherited. These *de novo* mutations cause many well-known genetic diseases.
We studied newly arising mutations that cause Apert syndrome, achondroplasia, multiple endocrine neoplasia 2B and Noonan syndrome in the testes of normal men. These conditions arise sporadically each generation at frequencies ranging from 1/2,000 to 1/100,000 live births and share common features. 1) The frequency of new cases due to spontaneous mutation is 100-1,000 fold higher than the highest known genome-average mutation rate. 2) A single mutated gene copy inherited by a child can cause the disease. 3) New mutations always arise in the unaffected father. 4) Older fathers are at greater risk for having affected children (paternal age effect). We introduced the term RAMP for diseases with these features, which is an acronym for Recurrent mutations, Autosomal dominant inheritance, Male-biased mutation and Paternal age effect.
The unexpectedly high frequency of offspring with a new disease mutation might result from the DNA site being more susceptible to mutation (a hot spot). The frequency of spermatogonial stem cells (SSC) that

acquire a new mutation over a man's life would increase explaining the paternal age effect. We studied the above RAMP mutations in the testes of unaffected men using a testis dissection and mutation detection approach. We rejected the hot spot hypothesis for each disease mutation. Instead our data were consistent with normal SSC rarely undergoing any one of these RAMP mutations but, when they do, they acquire a proliferative advantage over the non-mutated SSC. This advantage increases the frequency of sperm carrying the mutated allele and the risk that a father will have an affected child as he ages. It is surprising that some mutations that have a selective advantage in the testis might reduce the fitness of those individuals who inherit it.

I will discuss the evidence to support these assertions. I will also suggest plausible molecular mechanisms that might explain the selective advantage of the mutated spermatogonial stem cells based on what is known about mouse and human spermatogenesis.

SUNDAY, APRIL 6, 2014
4:45 p.m. – 5:30 p.m.

LECTURE II

NOVEL SPERMATOGENIC PATHWAYS AND MALE CONTRACEPTION

Martin M. Matzuk, MD, PhD, Denise Archambeault, PhD, Julio Castaneda, PhD, Zhifeng Yu, PhD, Mary Titus, Ryan Matzuk, Julio Agno, Ramiro Ramirez-Solis, PhD, James Bradner, MD and Masahito Ikawa, PhD

Baylor College of Medicine, The Wellcome Trust Sanger Institute, Dana Farber Cancer Institute and Harvard Medical School, and Osaka University

Objectives: Over the last two decades, our research program has focused on the identification and functional analysis of genes and pathways involved in mammalian reproduction. In the process, we have identified novel genes involved in germ cell intercellular bridge formation (*e.g.,* TEX14) and the piRNA pathway (*e.g.,* GASZ). Infertility in male mice lacking a specific gene would indicate that the gene product would be a novel target for contraception in men. Our goals are to identify and characterize germ-cell specific genes required for fertility and determine if these proteins are druggable targets for contraception.

Methods: We have taken a discovery-based approach to uncover male fertility required genes and small molecules that target these essential proteins for a reversible contraceptive effect.

Results: We have already published papers on some of the novel testis-specific gene products, and in 2012, we showed that JQ1 can target BRDT for a reversible contraceptive effect in mice.

Conclusions: Our discovery-based approach has opened up new avenues of research in our laboratory and the fields of infertility and contraception. We believe that germline-specific proteins are excellent targets for reversible contraception in men.

Funding for our research has been provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

MONDAY, APRIL 7, 2014
8:00 a.m. – 9:00 a.m.

WOMEN IN ANDROLOGY LECTURE

HORMONE SIGNALING AND REPROGRAMMING IN HUMAN PROSTATE STEM CELLS

Gail S. Prins, PhD
Department of Urology, University of Illinois at Chicago

Early-life exposures to estrogens reprograms the rat prostate gland structure and epigenome leading to differentiation defects and increased susceptibility to cancerous lesions with aging (1). We hypothesize that developmental estrogenization of the prostate occurs, in part, through stem and progenitor cell reprogramming that permits long-term memory of this exposure throughout life. To address whether this occurs in humans, we developed *in vitro* and *in vivo* models utilizing cells from organ donors as well as human embryonic stem cells (hESC). Stem and progenitor cells were isolated from primary prostate epithelial cells (PrEC) of young, disease-free donors using FACS and 3-D prostasphere (PS) culture. Studies confirmed both cell populations as ERα$^+$ and ERβ$^+$ and showed that estradiol-17□ (E_2) significantly increased their proliferation. The estrogenic endocrine disruptor, bisphenol A (BPA), likewise increased stem-progenitor cell self-renewal and stem-related gene expression. Further, findings identify reprogrammed genes and sncRNAs in prostate progenitor cells with E_2 and low-dose BPA exposure suggesting epigenetic reprogramming. While E_2 initiated genomic ERE signaling, both E_2 and BPA activated membrane ERs with rapid induction of p-Akt and p-Erk. Additional studies identified distinct roles for ERs with ERα driving stem cell self-renewal and ERβ promoting stem cell entry into a differentiation pathway.

An *in vivo* model to assess carcinogenicity was developed using human PS cells mixed with rat UGM and grown as renal grafts in nude mice, forming normal human prostate epithelium at one month. Exposure to E_2+T for 2 – 4 months led to PIN or PCa at low incidence (13%). Developmental BPA exposure was modeled by daily feeding of hosts for two weeks after grafting (0.39-1.35 ng free-BPA/ml serum). Upon E_2+T for 2 – 4 months, the PIN/PCa incidence increased ($P<0.01$) to 33-36%. Similar modeling utilizing hESC reveals that BPA can augment prostate stem-cell self-renewal and is sufficient drive prostate pathology in the mature human prostate epithelium. Together these findings indicate that early stage progenitor and stem cells in the human prostate are direct E_2 and BPA targets and that developmental BPA exposure reprograms the human prostate epithelium leading to elevated PCa susceptibility.

1. Prins GS and Ho SM: Early life estrogens and prostate cancer in an animal model. *Journal of Developmental Origins of Health and Disease*, 1 (6): 365-370, 2010.

SPEAKER ABSTRACTS

MONDAY, APRIL 7, 2014
9:15 a.m. – 10:15 a.m.

SYMPOSIUM II – Would You Give This Many Testosterone? Case Based Discussion

J. Lisa Tenover, MD, PhD and Peter N. Schlegel, MD[1]
Stanford University, Stanford, CA; [1]Weill Cornell Medical College, New York, NY

A decision by healthcare providers to give testosterone replacement therapy to an older male patient should rely on careful consideration of the potential benefits and risks of such therapy. Each patient, however, offers has at least a subtly different clinical presentation, so weighing the relative benefits and risks for a specific patient is not always straightforward. The clinical evidence to support testosterone replacement for older men are relatively limited. During this symposium, several brief clinical cases will be presented to highlight some typical clinical situations. Each case will be followed by a review of the current literature as it pertains to the treatment issues being considered. Cases scenarios will include management of a hypogonadal man with cardiovascular disease, who has both fatigue and decline in physical function. We will also discuss treatment considerations for a hypogonadal man with erectile dysfunction who also has a history of radical prostatectomy for prostate cancer.

MONDAY, APRIL 7, 2014
1:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility

QUANITATIVE AND QUALTITATIVE ASPECTS OF THE HORMONAL CONTROL OF SPERMATOGENESIS REVISITED

Ilpo Huhtaniemi, MD, PhD, FMedSci, Olayiwola Oduwole, PhD and Hellevi Peltoketo, PhD
Institute of Reproductive and Developmental Biology, Hammersmith Campus, Imperial College London, London W12 0NN, UK

The manipulation of gonadotropin action in genetically modified mice has provided us with novel information about qualitative and quantitative aspects of the hormonal control of spermatogenesis. We tested in the hypogonadal luteinizing hormone receptor knockout (LuRKO) mouse the concept of the hormonal male contraception, i.e. that a single dose of testosterone (T) supplementation can suppress gonadotropins and testicular T production while simultaneously maintaining extragonadal sexual and anabolic androgen actions. It was found that the dose-responses of all extragonadal and intragonadal actions of T were practically identical. Hence, a single dose of T that would produce suppression of gonadotropin and testicular T production without simultaneously turning on spermatogenesis could not be defined. This explains why the hormonal male contraception with T has insufficient efficacy. In another study we crossed the LuRKO mice with a transgenic mouse expressing a constitutively activated mutant of follicle-stimulating hormone receptor (FSHR-CAM). While the LuRKO mice are azoospermic, the FSHR-CAM mutant males have no apparent phenotype. Interestingly, the LuRKO/FSHR-CAM double mutants had normal spermatogenesis. This was initially interpreted to be due to stimulation of Leydig cell T production by Sertoli cell-derived paracrine factors stimulated by enhanced FSHR function. However, spermatogenesis persisted in the double mutant mice when they were treated with antiandrogen (flutamide). This indicated that missing androgen stimulation of spermatogenesis can be compensated for by enhanced FSH action. Hence, it appears that T and FSH have additive and complementary effects on spermatogenesis. It was shown earlier that FSH/FSHR knockout male mice have largely normal spermatogenesis. Here we demonstrate that enhanced FSH stimulation can compensate for the absence of androgens in the maintenance of spermatogenesis.

MONDAY, APRIL 7, 2014
1:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility

CA2+ AND CAMP SIGNALING CROSSTALK DURING SPERM CAPACITATION

Pablo Visconti, PhD
Department of Veterinary and Animal Sciences. University of Massachusetts, Amherst.

Mammalian sperm become fertilization competent in the female tract in a process known as capacitation. This process is correlated with functional changes in sperm parameters such as the activation of sperm motility known as hyperactivation and the preparation to undergo a physiologically induced acrosome reaction. Taking into consideration the highly differentiated and compartmentalized nature of sperm, it can be postulated that the molecular basis of capacitation should account for independent changes occurring in different sperm compartments such as the flagellum (e.g. hyperactivation) and the head (e.g. preparation for the acrosome reaction). At the molecular level, capacitation is associated with the activation of a PKA-dependent phosphorylation cascade and with hyperpolarization of their membrane potential. It has been shown in multiple species that activation of PKA is needed for hyperactivation and to prepare the sperm for the acrosome reaction. Capacitation is also associated with the increase in intracellular Ca^{2+} concentrations. Work from our laboratory indicates that there is a crosstalk between the cAMP and the Ca^{2+} pathway. On one hand Ca^{2+} regulates cAMP synthesis and also its degradation. On the other hand, cAMP and PKA are upstream of the increase in Ca^{2+} needed for hyperactivation and for the sperm to acquire fertilizing capacity.

MONDAY, APRIL 7, 2014
1:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility

AGING AFFECTS GERM CELLS FROM GENES TO FERTILITY

Bernard Robaire, PhD
Departments of Pharmacology & Therapeutics and of Obstetrics and Gynecology, McGill University, Montreal, Canada

The age of paternity is increasing and there is growing societal concern regarding the potential consequences of this increase to progeny. Several epidemiological studies have established clear links between paternal age and an increased incidence of conditions such as autism, diabetes, cardiovascular anomalies, and schizophrenia in the next generation. Using animal studies, we have found that increasing paternal age affects progeny outcome, sperm quality, and the response to oxidative stress. We found significantly altered expression of genes involved in DNA damage/repair, the response to oxidative stress, and cell adhesion in isolated pachytene spermatocytes, but not in round spermatids, from young and aged rats. Further analysis of pachytene spermatocytes demonstrated that genes involved in the base excision repair (BER) and nucleotide excision repair (NER) pathways were specifically altered during aging. These studies established that aging is associated with differential reg-

ulation of DNA repair pathways. Furthermore, in aged males there was an increase in 8-oxo-2´-deoxyguanosine (8-oxodG) immunoreactivity in the testes and in the number of spermatozoa positive for 8-oxodG; thus, downregulation of the BER pathway led to oxidative-stress related deficient repair of 8-oxo-dG lesions in germ cells. We also found changes in the expression of over 70 transcripts involved in cell adhesion; of these, at least 20 are specifically involved in junction dynamics within the seminiferous epithelium. The mRNAs and proteins of many cell adhesion/junction markers were decreased by at least 50% in aged spermatocytes. We saw a gradual collapse of the blood-testis barrier between 18 and 24 months. The damage to spermatogenic cells from aged rats led us to hypothesize that spermatogonial stem cells may be affected. Using CD9+ enriched GFP-marked spermatogonial cells from young and aged rats and transplanting them into the testes of busulfan-treated nude mice, we found that both colony numbers and size were affected by age. The transcriptomes of FACS-isolated spermatogonial cells were analyzed to evaluate molecular changes occurring in these cells with age. In the aged CD9+ enriched cells, an altered gene expression was found for transcripts involved in mitosis and in DNA damage response. These molecular alterations in the spermatogonial enriched population of cells from the testes of aged rats imply that stem/progenitor spermatogonia are contributors to the germ cell origin of reproductive aging.

These studies were funded by the Canadian Institutes for Health Research.

MONDAY, APRIL 7, 2014
3:30 p.m. – 4:15 p.m.

LECTURE III

THE STRESS HORMONE CORTICOTROPIN-RELEASING FACTOR ACTS IN THE BRAIN AND THE TESTES TO REGULATE TESTOSTERONE SECRETION

Catherine Rivier, PhD
The Salk Institute for Biological Studies, La Jolla, CA

Objectives: Testosterone (T) secretion is usually considered hormonally regulated by hypothalamic gonadotropin-releasing hormone (GnRH), the ensuing secretion of LH and the feedback provided by testicular steroids. However, dissociated LH and T release is observed under a variety of stressors. This led us to propose the existence of a multisynaptic neural pathway between the brain and the testes, independent of the pituitary that inhibits T secretion. Evidence for this pathway was further indicated by the ability of intracerebroventricularly (icv) administered corticotropin-releasing factor (CRF) or monoamines, to block the T response to hCG.
Methods: We injected the retrograde tracer pseudorabies virus (PRV) into the testes, lesioned specific sites of the proposed circuit and identified the brain regions of the proposed pathway by double labeling with PRV, CRF and/or and tyrosine hydroxylase (TH).
Results: PRV staining was found in the spinal cord, the locus coeruleus (LC) and the paraventricular nucleus (PVN) of the hypothalamus. Co-labelling of CRF and PRV was found in the PVN, and co-labelling of PRV and TH in the PVN, the LC and the ventral norepinephrine pathway of the brain stem. Spinal cord transection at T7-T8 prevented brain staining, and restored hCG-induced T release in rats injected with CRF or monoamines icv. The inhibition of these icv treatments is not due to sympathetically-mediated vasoconstriction of, or decreased blood flow to the testis, and is mimicked by their microinfusion into the PVN. CRF, isoproterenol or alcohol also decreased testicular levels of the steroidogenic acute regulatory protein and the peripheral-type benzodiazepine receptor.
Conclusions: We propose that in the male rat, Leydig cell function depends on both a fast, pituitary-independent neural pathway, as well as a slower hormonal pathway represented by the classical hypothalamic GnRH/pituitary LH connection. CRF and catecholamines may act as neurotransmitters in the brain-testicular circuit. Alcohol and other stressors may inhibit male reproductive functions not only through their known effects on hypothalamic GnRH and/or pituitary LH, but also through the proposed neural circuit.

Funding provided by by NIH grant AA 12810.

MONDAY, APRIL 7, 2014
4:15 p.m. – 5:00 p.m.

LECTURE IV

PHARMACOLOGICAL REGULATION OF STEROID BIOSYNTHESIS: FROM TESTIS TO BRAIN

Vassilios Papadopoulos, PhD
The Research Institute of the McGill University Health Centre and the Department of Medicine, McGill University, Montreal, Quebec, Canada

Gonadal and adrenal steroidogenesis are increased by pituitary hormones which accelerate the delivery of the substrate cholesterol from intracellular stores to mitochondrial CYP11A1. Placenta and brain make steroids in a hormone-independent manner, in the case of placenta to satisfy fetal-maternal requirements, and in the case of brain to form small amounts locally needed to control neuronal function. Considering the role of steroids as mediators of development, reproduction, body homeostasis, adaptation and behavior, it is obvious that changes in the rate of steroid formation could result in pathological states. In the testis, reduced serum testosterone (T) is common among subfertile and infertile young men. Reduced T is also common in aging men and is often associated with mood changes, fatigue, depression, decreased lean body mass, metabolic syndrome, and reduced sexual function. Although T-replacement therapy has been the treatment of choice in both young and aging men, the undesired side-effects associated with flooding the body with large amounts of T drove the search for the development of repair therapies designed to restore the ability of the testis itself to make T. In contrast, in the case of excessive steroid production associated with Leydig cell tumors, inhibitors of steroid formation might be used to control the rate of excessive steroid synthesis. In the brain, steroids have both long-term and rapid effects, acting as local regulators of neural development and excitability. Changes in neurosteroid levels are linked to the development of neuropsychiatric and neurological disorders such as depression, anxiety and neurodegeneration. Local administration of neurosteroids is unfeasible, and treatment of patients with large amounts of neuroactive steroids is unsafe. Thus, there is a clear need for developing repair therapies that restore the brain's ability to make neurosteroids. Progress in the development of compounds that target proteins involved in cholesterol transport into mitochondria in the testis and brain, and in this way help to control steroid biosynthesis in these organs, will be discussed.

SPEAKER ABSTRACTS

TUESDAY, APRIL 8, 2014
8:00 a.m. – 9:15 a.m.

SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?

PSA AND PROSTATE CANCER SCREENING DEBATE

William J. Catalona, MD
Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

The US Preventive Services Task Force (USPSTF) and American Urological Association (AUA) guidelines take steps in the wrong direction for patient-centered care and, if implemented, would deprive many men of the opportunity to pursue shared decision-making about life-saving PSA testing. A more forward-looking approach is needed.

These guidelines are based on incomplete data and inaccurate estimates of the benefits and harms of PSA testing. Guidelines panels rely on evidence from randomized clinical trials (RCTs) and statistical modeling studies, but the available RCTs provide little reliable evidence, and some are profoundly flawed. Many medical organizations have reviewed the same body of evidence and formulated vastly divergent guidelines, ranging from the USPSTF recommending *against* PSA testing for any man, to the European Association of Urology recommending a baseline PSA test beginning at age 40-45, with follow-up testing for all men with a life expectancy of ≥10 years, always with shared decision making between the man and his doctor.

The RCTs were conducted over a limited time period and do not reveal true information about absolute benefits of screening over a lifetime. The use of RCT data to estimate benefits and harms of PSA testing underestimates benefits and exaggerates harms. In assessing benefits, the USPSTF and AUA panels focused solely on prostate cancer death without considering avoiding suffering from metastases that might not have resulted in a cancer death. An analogy would be a study of the benefits of wearing seatbelts in cars. Is the benefit only the deaths prevented, or should it also include the catastrophic injuries prevented that did not result in death? Avoiding metastases significantly shifts the balance of harms and benefits, as men diagnosed with metastases ultimately require more treatments and have more side effects.

In assessing the harms of testing, the panels cast a net over a variety of side effects of PSA testing, biopsy, and treatment that range from minor to serious. The possible harms of a simple blood test should not be linked with those of biopsy and treatment, and few of these side effects reach the extreme of a prostate cancer death.

The AUA guidelines do not recommend screening men <55 years old with an average risk of prostate cancer. The primary objective of baseline testing in men in their 40s is to assess the risk for subsequent life-threatening prostate cancer. Men in their 40s in the top 10% of PSA levels for their age group account for almost half of all prostate cancer deaths up to 30 years later, and those with levels above 1 ng/mL warrant more careful monitoring. A high baseline PSA in a man in his 40s is a stronger risk factor than African heritage or a positive family history. It is impossible to fully assess whether a man is at high risk without measuring a baseline PSA in early middle age.

The AUA did not recommend testing men <55 years is that the RCTs have not adequately tested PSA screening in this age group. The available evidence suggests it is beneficial. Starting testing at age 55 is too late. There is no reason to believe that if PSA testing works in men 55 to 69 years old, it would not also work in men 45-55 years old. Although the AUA guidance document explains that the panel does not recommend *against* PSA testing for men 40- 55 years old, the actual guidelines statement uses the language, "we do not recommend." Rather, it should read, "there is insufficient evidence to recommend *for or against* early detection in men younger than 55."

The AUA panel's suggestion for longer testing intervals needs to be reconciled with the realization that less frequent testing limits the ability to detect aggressive cancers that have the shortest preclinical phases and that, with less frequent testing, there remains the undesirable effect of detecting all of the low-risk cancers (length-time bias), possibly doing more harm than good.

The AUA also does not recommend routine testing in men >69 years old, despite the fact that 50% of prostate cancer deaths occur in men diagnosed after age 75. Age 70 is too young to stop testing in healthy men who have a 10-15 year life expectancy. Therefore, testing in men over 70 should be performed on an individual basis with shared decision-making. In the absence shared decision making, men are more than twice as likely *not* to undergo testing.

There has been a 75% reduction in metastatic disease at the time of prostate cancer diagnosis and more than a 45% decrease in the age-adjusted prostate cancer mortality rate in the U.S. during the PSA era, largely attributable to PSA testing. Similar trends have been observed in other countries where PSA testing is widely practiced. Restricting PSA testing too much would significantly compromise these benefits.

TUESDAY, APRIL 8, 2014
8:00 a.m. – 9:15 a.m.

SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?

CHOOSING WISELY ABOUT PSA TESTING: WHY SAYING "NO" IS A GOOD HEALTH-CARE CHOICE

Timothy Wilt, MD, MPH

Questions remain whether PSA screening and subsequent early treatment for screen detected prostate cancer provides lifetime benefits that exceed harms. Yet, PSA screening for prostate cancer is common. However, current data indicate that this balance is not favorable, especially as currently practiced in the U.S. through at least 15 years and results in large health care costs. Implementation of high value prostate cancer care requires a change in practice through science-based educational and policy initiatives. A review of the goals of cancer screening strategies, best evidence regarding the main benefits and harms of prostate cancer screening, current prostate cancer screening recommendations as well as the principals and ethics of high-value care will be presented. I will provide suggestions on guiding clinicians in implementation of high-value prostate cancer care and helping their patients to choose wisely about PSA testing.

SPEAKER ABSTRACTS

TUESDAY, APRIL 8, 2014
8:00 a.m. – 9:15 a.m.

SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?

TARGETED APPROACH TO PROSTATE SPECIFIC ANTIGEN (PSA) BASED PROSTATE CANCER DETECTION: THE RATIONAL CHOICE

H. Ballentine Carter, MD
Johns Hopkins School of Medicine

Objectives: Review the rationale for a targeted approach to prostate cancer screening using prostate specific antigen (PSA) to assess risk.
Methods: A systematic literature review was commissioned by the American Urological Association (AUA) to inform the practice of prostate cancer detection. A methodology team reviewed over 300 studies that evaluated outcomes important to patients (prostate cancer, incidence/mortality, quality of life, diagnostic accuracy and harms of testing). A multidisciplinary panel (general internal medicine, cancer epidemiology, health policy, and medical, radiological and urological oncology) interpreted the evidence and formulated statements to assist the clinician and the *asymptomatic average risk* man in decision-making regarding prostate cancer detection.
Results: There was no evidence to address the outcomes of interest to patients other than with PSA based prostate cancer screening. PSA based screening in the US was estimated to have contributed approximately 50 percent of the overall 40 percent reduction in prostate cancer mortality that occurred over the last two decades. This would be consistent with the decline in prostate cancer mortality reported in randomized prostate cancer screening trials in which there was minimal contamination of controls and low prescreening rates. However, an approach to screening that assumes that benefits will be shared equally among all ages and risk groups (non targeted), and results in treatment of most individuals after diagnosis regardless of cancer aggressiveness, resulted in over treatment rates that are estimated to be 30 percent or more. Thus, a more targeted screening approach is necessary to reduce over treatment of prostate cancer and is supported by the AUA. The strongest evidence that benefits may outweigh harms was in men age 55-69 years undergoing PSA based screening. This led the panel to recommend shared decision making for these men at average risk, but recommend against *routine* screening for other age groups at average risk. Further, to reduce the harms associated with screening (false positive tests, over diagnosis, over treatment), the panel recommended against annual screening for those who choose to be screened.
Conclusions: A panel under the auspices of the AUA recommended a targeted approach to PSA based screening that involves shared-decision making for the average risk asymptomatic man between ages 55-69 years.

TUESDAY, APRIL 8, 2014
9:15 a.m. – 10:15 a.m.

INTERNATIONAL LECTURE

PHARMACOGENETICS OF FSH

Manuela Simoni, MD, PhD
Unit and Chair of Endocrinology, Dept of Biomedicine, Metabolic and Neural Sciences, University of Modena & Reggio Emilia, Modena, Italy.

Objectives: FSH acts through its receptor, the FSHR on Seroli cells. Recently, several single nucleotide polymorphisms (SNP) were found to be associated both with biological parameters of FSH action and with the pharmacological response to FSH. Here, I will assess the potential pharmacogenetic use of FSH for infertility treatment.
Methods: Critical review of the literature and genomic databases. In vitro experiments, using human granulosa-lutein cells and transiently transfected COS7 cells. Study of the activated signal transduction pathways by Western Blotting. SNP assessed: rs6166 (c.2039A>G, p.N680S), rs6165 (c.919A>G, p.T307A), rs1394205 (c.-29G>A) in *FSHR* and rs10835638 (c.-211G>T) in *FSHB*. Literature search via PubMed. Blast analysis of genomic information available in the NCBI nucleotide database. Prospective, randomized clinical trial assessing FSH effects on sperm DNA fragmentation index in idiopathic infertile men with oligo-asteno-theratozoospermia selected according to their *FSHR* genotype.
Results: All SNPs appear first in *Homo*, result in reduced FSH action and are present with variable frequencies and combinations worldwide. Stringent clinical studies demonstrate that the *FSHR* genotype influences serum FSH levels and gonadal response. Serum FSH levels depend on the -211G>T SNP, influencing transcriptional activity of the *FSHB* promoter. Genotypes reducing FSH action are overrepresented in infertile subjects. The response to FSH in infertile men depends on the *FSHR* genotype
Conclusions: Considering the combination of *FSHR* and *FSHB* genotypes has the potential for a much stronger clinical impact than either one alone. About 20% of people are carrier of the alleles associated with lower serum FSH levels/reduced *FSHR* expression or activity, possibly less favorable for reproduction. Prospective studies need to investigate whether stratification of infertile patients according to their *FSHR-FSHB* genotypes improves clinical efficacy of FSH treatment compared to the current, naïve approach. A relative enrichment of less favorable *FSHR-FSHB* genotypes may be related to changes in human reproductive strategies and be a marker of some health-related advantage at the costs of reduced fertility.

SPEAKER ABSTRACTS

TUESDAY, APRIL 8, 2014
10:30 a.m. – 12:00 p.m.

SYMPOSIUM V – Innovations in Male Environmental Health Protection

REVOLUTION IN TOXICITY TESTING AND RISK PREDICTION FOR CHEMICALS IN THE ENVIRONMENT

Thomas Knudsen, PhD
US EPA/ORD/NCCT, Research Triangle Park, NC

Addressing safety aspects of drugs and environmental chemicals relies extensively on animal testing; however, the quantity of chemicals needing assessment and challenges of species extrapolation require alternative approaches to traditional animal studies. Newer in vitro and in silico approaches focus on predictive modeling of adverse outcome pathways (AOPs) using computational and high-throughput screening (HTS) data for thousands of chemicals and hundreds of HTS assays in EPA's ToxCast inventory. Virtual Tissue Models (VTMs) built for developmental processes simulate multiscale disruptions in the system and provide a quantitative spatio-temporal prediction of how chemicals might impact embryo-fetal development. Virtual embryo models integrate empirical data with embryological information to simulate dynamic biological tissue architectures relevant to specific AOPs. This approach is being used to evaluate chemical effects on development, such as disruption of blood vessel formation (angiodysplasia), palatal fusion (cleft palate), limb outgrowth (ectrodactyly) and urethral fusion (hypospadias) among other systems. Simulations of endocrine and vascular pathways can be parameterized in this way, using in vitro data for chemical prioritization and early lifestage exposure considerations.
This work was funded by the US EPA under its Chemical Safety for Sustainability Research Program but does not reflect US EPA policy.

TUESDAY, APRIL 8, 2014
10:30 a.m. – 12:00 p.m.

SYMPOSIUM V – Innovations in Male Environmental Health Protection

RESPONSE OF HUMAN FETAL TESIS XENOTRANSPLANTS TO ENVIRONMENTAL TOXICANTS: IMPLICATIONS FOR RISK ASSESSMENT

Kim Boekelheide, MD, PhD, and Daniel J Spade, PhD
Brown University

Objectives: Male rats exposed in utero during critical periods of reproductive development to an active phthalate, such as di-n-butyl phthalate (DBP), have alterations in the developing testis, including effects on the seminiferous cords and suppressed Leydig cell steroidogenesis. Interestingly, however, male mice similarly exposed in utero are resistant to the anti-androgenic effects of phthalates. This study used human fetal testis xenotransplants to determine the response of human fetal testis to phthalates.
Methods: Adult male athymic nude mice were castrated, and human fetal testis fragments (gestational week 16-22) were xenografted into the renal subcapsular space. Hosts were treated with human chorionic gonadotropin for 4 weeks to stimulate testosterone production. During weeks 3 and 4, hosts were exposed to DBP (500 mg/kg/d po) or abiraterone acetate (75 mg/kg/d po), a potent irreversible CYP17A1 inhibitor.
Results: Abiraterone acetate significantly reduced host testosterone and the weights of androgen-sensitive host organs, while DBP had no effect on androgenic endpoints. DBP produced a near-significant increase in multinucleated germ cells in the xenografts, an indication of an effect upon seminiferous cords.
Conclusions: We have developed an assay, similar to the Hershberger assay, that evaluates human fetal testis for anti-androgenic effects of environmental toxicant exposure. Abiraterone acetate dramatically reduced steroidogenesis in human fetal testis xenografts. Similar to the mouse, but unlike the rat, 500 mg/kg/d DBP had no effect on human fetal testis testosterone production. These results provide novel, human-relevant mechanistic insight into the effects of phthalates on the developing male reproductive tract.
Funding: Supported by grants from the National Institute of Environmental Health Sciences of the National Institutes of Health (R01 ES017272 to KB, T32 ES007272 to DJS).

TUESDAY, APRIL 8, 2014
10:30 a.m. – 12:00 p.m.

SYMPOSIUM V – Innovations in Male Environmental Health Protection

TRANSLATION OF THE SCIENCE IN MALE REPRODUCTIVE AND ENVIRONMENTAL HEALTH FOR EVIDENCE-BASED DECISIONS BY CLINICIANS, REGULATORS AND THE PUBLIC

Paula I. Johnson, Patrice Sutton and Tracey J. Woodruff
Program on Reproductive Health and the Environment, University of California - San Francisco

Patient exposure to toxic environmental chemicals is ubiquitous, and preconception and prenatal exposures can have a profound and lasting impact on reproductive health across the life course. Organizations such as the American Congress of Obstetricians and Gynecologists and the Endocrine Society have called for timely action to prevent harm. In the clinical sphere systematic reviews are used to inform risk/benefit decisions for patient care. However, due to differences in the evidence stream and decision context, there is no established corollary to making recommendations about environmental exposures. Beginning in 2009, a collaboration of 22 clinicians and scientists developed the Navigation Guide systematic review methodology, modeled after best practices in evidence-based medicine and environmental health science. As part of proof of concept we have applied the Navigation Guide methodology to the question: What is the impact of exposure to the antimicrobial triclosan on male reproductive health? We adapted established clinical medicine and healthcare quality and risk of bias tools to assess individual studies and to rate the quality and strength of an entire body of evidence for toxicity. The adoption of an efficient systematic and transparent method will speed the incorporation of research into clinical and policy decision-making to protect patient and public health.
The development of the Navigation Guide methodology and proof-of-concept was funded by grants from New York Community Trust, California Environmental Protection Agency, Clarence Heller Foundation, Passport Foundation, Heinz Endowments, Fred Gellert Foundation, Rose Foundation, Kaiser Permanente, UC San Francisco Institute for Health Policy Studies, Planned Parenthood Federation of America, National Institute for Environmental Health Sciences (ES018135), US Environmental Protection Agency EPA STAR (RD83467801) and USEPA through a contract with Abt Associates (GAIA-0-6-UCSF 17288), and appointments to the Internship/Research Participation Program at the National Center for Environmental Economics, USEPA, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and EPA.

*POSTER SESSION I

**Not CME Accredited*
Sunday, April 6, 2014
11:00 a.m. – 12:30 p.m.
Location: Venetian

Poster# 1 **LONG-TERM TREATMENT WITH TESTOSTERONE UNDECANOATE (TU) IN HYPOGONADAL MEN WITH CARDIOVASCULAR DISEASES (CVD): OBSERVATIONAL DATA FROM A REGISTRY STUDY**
Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Gheorghe Doros, PhD[3] and Abdulmaged Traish, PhD[4]
[1]Bayer Pharma AG, Global Medical Affairs Andrology; [2]Private Urology Practice; [3]Boston University School of Public Health; [4]Boston University School of Medicine
(Presented By: Farid Saad, DVM, PhD)

Poster# 2 **156 HYPOGONADAL MEN WITH OBESITY AND TYPE 2 DIABETES ACHIEVE WEIGHT LOSS AND IMPROVED GLYCAEMIC CONTROL UPON TREATMENT WITH TESTOSTERONE UNDECANOATE UP TO 6 YEARS: A SUBGROUP ANALYSIS FROM TWO OBSERVATIONAL REGISTRY STUDIES**
Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Aksam Yassin, MD, PhD[3], Gheorghe Doros, PhD[4] and Abdulmaged Traish, PhD[5]
[1]Bayer Pharma AG, Global Medical Affairs Andrology; [2]Private Urology Practice; [3]Institute for Urology and Andrology; [4]Boston University School of Public Health; [5]Boston University School of Medicine
(Presented By: Farid Saad, DVM, PhD)

Poster# 3 **LACK OF ACTIVATION OF ENCLOMID TO ITS 4-HYDROXYLATED FORM BY CYP 2D6 DOES NOT EXPLAIN LACK OF TESTOSTERONE RESPONSE**
Ronald Wiehle, PhD, Gregory Fontenot, PhD and Kuang Hsu, BS/MS
Repros Therapeutics Inc., The Woodlands TX 77381
(Presented By: Ronald Wiehle, PhD)

Poster# 4 **ESTRADIOL INCREASES THE PROLIFERATION OF RAT IMMATURE LEYDIG CELLS: A POSSIBLE ROLE FOR LEYDIG CELL TUMOR FORMATION**
Xiaoheng Li, Haiyun Deng, PhD, Xiaomin Chen, PhD, Kaimin Yuan, PhD, Ying Su, MS, Shiwen Liu, MS, Tiao Bu, MS, Qingquan Lian, PhD, Ren-Shan Ge, PhD and Guimin Wang, PhD
The 2nd Affiliated Hospital& Research Academy of Reproductive Biomedicine, Wenzhou Medical University, Wenzhou, Zhejiang 325027, China
(Presented By: Xiaoheng Li)

Poster# 5 **PLATELET-DERIVED GROWTH FACTOR (PDGF) STIMULATES DIFFERENTIATION OF RAT IMMATURE LEYDIG CELLS VIA INCREASING THE EXPRESSION OF STAR**
Xiaomin Chen, PhD[1], Xiaoheng Li, MS[2], Kaimin Yuan, PhD[3], Shiwen Liu, MS[4], Tiao Bu, MS[4], Qiufan Wang, PhD[5], Qingquan Lian, PhD[5], Ren-Shan Ge, PhD[5] and Guimin Wang, PhD[5]
[1]Research assistant; [2]Laboratory technician; [3]Attending doctor; [4]Master student; [5]Professor
(Presented By: Xiaomin Chen, PhD)

Poster# 6 **TESTOSTERONE AS PROGNOSTIC INDEX IN ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE**
Sebastiano Raimondo, Trainee[1], Alessandro Di Marco Berardino, Trainee[2], Chantal Di Segni, Trainee[1], Riccardo Inchingolo, MD[2], Andrea Smargiassi, MD[2], Salvatore Valente, MD[2], Giuseppe Maria Corbo, MD[2], Alfredo Pontecorvi, MD[1] and Antonio Mancini, MD[1]
[1]Dept. Of Medical Sciences, Division of Endocrinology, Catholic University of Sacred Heart, Rome; [2]Dept. Of Medical Sciences, Division of Pneumology, Catholic University of Sacred Heart, Rome
(Presented By: Antonio Mancini, MD)

Poster# 7 **INFLUENCE OF TESTOSTERONE DEPRIVATION ON OXIDATIVE STRESS INDUCED NEURONAL DAMAGE IN HIPPOCAMPUS OF ADULT RATS**
Prakash Seppan, PhD, Ganesh Lakshmanan, MSc, Karthik Ganesh Mohanraj, MSc, Venkata Lakshmi Nagella, MSc, Anuradha Murugesh, MSc and Dinesh Premavathy, MSc
University of Madras
(Presented By: Prakash Seppan, PhD)

POSTER SESSION I

Poster# 8 **EFFECTS OF FOUR CHEMOTHERAPEUTIC AGENTS, BLEOMYCIN, ETOPOSIDE, CISPLATIN AND CYCLOPHOSPHAMIDE, ON DNA DAMAGE AND TELOMERES IN A MOUSE SPERMATOGONIAL CELL LINE**

Mingxi Liu, PhD, Barbara Hales, PhD and Bernard Robaire, PhD
McGill University
(Presented By: Mingxi Liu, PhD)

Poster# 9 **EFFECT OF ROSMARINIC ACID ON SERTOLI CELLS APOPTOSIS AND SERUM ANTIOXIDANT LEVELS IN RATS AFTER EXPOSURE TO ELECTROMAGNETIC FIELDS**

Arash Khaki
(Presented By: Arash Khaki)

Poster# 10 **HUMAN SPERM BIOASSAY IN EVALUATING THE QUALITY OF BLOOD SERUM AND FOLLICULAR FLUID OF FEMALES UNDERGOING IN VITRO FERTILIZATION (IVF) BASED INFERTILITY TREATMENT**

Amjad Hossain, PhD
The University of Texas Medical Branch
(Presented By: Amjad Hossain, PhD)

Poster# 11 **EFFECTS OF APIGENIN ON THE DEVELOPMENT AND FUNCTION OF RAT IMMATURE LEYDIG CELLS**

Qiqi Zhu Master[1], Jian Jin Master[2], Dongxin Chen Bachelor[1], Shiwen Liu Bachelor[1], Tiao Bu Bachelor[1], Huina Su Bachelor[1], Feihua Wu Master[2], Qingquan Lian Doctor[1] and Ren-Shan Ge Doctor[1]
[1]The 2nd Affiliated Hospital & Institute of Reproductive Biomedicine, Wenzhou Medical University; [2]Department of Pharmacy, Shanghai No.9 People's Hospital, School of Medicine, Shanghai Jiao Tong University
(Presented By: Qiqi Zhu Master)

Poster# 12 **WITHDRAWN**

Poster# 13 **STIMULATION OF STEROIDOGENESIS IN RAT IMMATURE LEYDIG CELLS BY BROMINATED FLAME RETARDANT BDE-100**

Haiyun Deng, MD, Dongxin Chen, MS, Tiao Bu, MS, Siwen Liu, MS and Jingjing Guo, MS
The 2nd Affiliated Hospital & Research Academy of Reproductive Biomedicine
(Presented By: Haiyun Deng, MD)

Poster# 14 **MONONUCLEAR PHAGOCYTES FROM THE PROXIMAL MOUSE EPIDIDYMIS TAKE UP LUMINAL BACTERIA**

Tegan Smith, PhD, Jeremy Roy, PhD, Lubov Grigoryeva, BS, Sylvie Breton, PhD and Nicolas Da Silva, PhD
Massachusetts General Hospital/Harvard Medical School
(Presented By: Tegan Smith, PhD)

Poster# 15 **ROLE OF SPERM TRANSCRIPTS IN THE ETIOLOGY OF IDIOPATHIC RECURRENT EARLY PREGNANCY LOSS**

Kranthi Vemparala, PhD, Manoj Kumar, MSc, Shwetasmita Mishra, MSc and Rima Dada, MD, PhD
Molecular reproduction and Genetics lab, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Kranthi Vemparala, PhD)

GENETICS

Poster# 16 **PRIMARY TESTICULAR FAILURE: GENOTYPE PHENOTYPE CORRELATION OF 140 CASES**

Ashutosh Halder, MD, DNB, DM[1], Manish Jain, PhD[2] and Prashant Kumar, MSc[3]
[1]Additional Professor, Reproductive Biology, AIIMS; [2]Scientist, AIIMS, New Delhi; [3]PhD Student, AIIMS, New Delhi
(Presented By: Ashutosh Halder, MD, DNB, DM)

Poster# 17 **SPERM TELOMERE LENGTH AND DNA INTEGRITY: ROLE IN IDIOPATHIC MALE INFERTILITY: IMPACTS OF LIFE STYLE INTERVENTIONS**

Swetasmita Mishra, MSc[1], Rajeev Kumar, MD[2], Shiv Basant Kumar, MSc[1] and Rima Dada, MD, PhD[1]
[1]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy; [2]Department of Urology, AIIMS
(Presented By: Swetasmita Mishra, MSc)

POSTER SESSION I

Poster# 18 **INTEGRATIVE DNA METHYLATION AND GENE EXPRESSION ANALYSES IDENTIFIES DISCOIDIN DOMAIN RECEPTOR 1 (DDR1) ASSOCIATION WITH IDIOPATHIC NONOBSTRUCTIVE AZOOSPERMIA (NOA)**

Ranjith Ramasamy, MD, Alex Ridgeway, Josephine Addai, Jason Scovell, Larry Lipshultz, MD and Dolores Lamb, PhD
Department of Urology, Baylor College of Medicine
(Presented By: Ranjith Ramasamy, MD)

Poster# 19 **AFFECT OF OXIDATIVE STRESS AND SPERM DNA DAMAGE ON EARLY EVENTS OF CONCEPTION, INDICES OF EMBRYO GROWTH AND EMBRYO QUALITY IN COUPLES OPTING FOR IVF**

Monis Bilal Shamsi, MSc, PhD, Sweta Smita Misro, MSc and Rima Dada, MD, PhD
Laboratory for Molecular Reproduction and Genetics, All India Institute of Medical Science
(Presented By: Monis Bilal Shamsi, MSc, PhD)

Poster# 20 **DETECTING SPERM DNA FRAGMENTATION TO DISCRIMINATE BETWEEN FERTILE AND INFERTILE MEN**

Marta Cambi, Ilaria Natali[1], Biagio Olivito[2], Chiara Azzari[2], Gianni Forti[3], Elisabetta Baldi[3] and Monica Muratori[3]
[1]Sterility Center, Obstetric and Gynecology Unit, S.S. Cosma and Damiano Hospital, Pescia,Italy; [2]Department of Health Sciences, A. Meyer Children's Hospital, Florence, Italy; [3]Experimental and Clinical Biomedical Sciences Andrology Unit, University of Florence,Italy
(Presented By: Marta Cambi)

Poster# 21 **INFERTILITY, RECURRENT SPONTANEOUS ABORTIONS, CONGENITAL MALFORMATIONS AND CANCER: POINTS OF COMMON CAUSALITY**

Swetasmita Mishra, MSc[1], Kuldeep Mohanty, MSc[2], Kranthi Vemparala, PhD[2], Madhuri Tolahunase, MSc[2], Rajeev Kumar, MD[3] and Rima Dada, MD, PhD[2]
[1]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy; [2]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; [3]Department of Urology, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Swetasmita Mishra, MSc)

Poster# 22 **MITOCHONDRIAL COPY NUMBER VARIATION: NO CORRELATION WITH SPERM DEFECTS: IMPLICATIONS IN ART**

Swetasmita Mishra, MSc[1], Manoj Kumar, MSc[2], Kranthi Vemparala, PhD[2], Rajeev Kumar, MD[3], Neena Malhotra, MD[4] and Rima Dada, MD, PhD[2]
[1]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy; [2]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; [3]Department of Urology, All India Institute of Medical Sciences, New Delhi, India; [4]Department of Obstretics & Gynaecology, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Swetasmita Mishra, MSc)

Poster# 23 **THE ANALYSIS OF PATERNAL AGE ON INTRACYTOPLASMIC SPERM INJECTION OUTCOME**

Feng Jiang, MD[1], Xian-dong Peng, MD[1], Hua Chen, MD[1], Guo-Wu Chen, MD[1], Xiao-xi Sun, MD[1] and Weipeng Zhao, MD[1,2]
[1]Shanghai Jiai Genetics & IVF Institute, China-USA Center, 588 Fangxie Road, Shanghai 200011, China; [2]Genetics & IVF Institute, 3015 Williams Drive, Fairfax, VA 22031, USA
(Presented By: Feng Jiang, MD)

Poster# 24 **ACONITI LATERALIS PREPARATA RADIX IMPROVES SPERM MOTILITY THROUGH UP-REGULATION OF THE CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) PROTEIN IN CYCLOPHOSPHAMIDE-TREATED MALE MICE**

Kyu jin Jung, MS, Kwan Suk Bang, PhD, Do Rim Kim, PhD, Ha Young Kim, MS, Eun Bit Ko, MS, Kyung Jun Shim, MS, Mun Seog Chang, PhD and Seong Kyu Park, PhD
Department of Prescriptionology, College of Korean Medicine, Kyung Hee University
(Presented By: Seong Kyu Park, PhD)

Poster# 25 **STUDY ON CONTRACEPTIVE EFFECT OF ETHANOL EXTRACTED JUSTICIA GENDARUSSA BURM.F. LEAVES IN FERTILE MEN: PHASE II CLINICAL TRIAL**

Bambang Prajogo, EW, PhD[1], Dyan Pramesti, MD, Master[2] and Sri Musta'ina, Master[2]
[1]Dept. Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University; [2]Reproductive Health Research Centre, School of Medicine Airlangga University
(Presented By: Dyan Pramesti, MD, Master)

POSTER SESSION I

Poster# 26 **ADVERSE EFFECTS OF CLOMIPHENE CITRATE IN INFERTILE MEN**

Mary Samplaski, MD[1], Tyler Gray, MD[2], Keith Jarvi, MD[2], Ethan Grober, MD[2] and Kirk Lo, MD[2]
[1]Mount Sinai Hospital, University of Toronto, Toronto, Ontario; [2]Mount Sinai Hospital, University of Toronto
(Presented By: Mary Samplaski, MD)

Poster# 27 **COCAINE USE IN THE INFERTILE MALE POPULATION: EFFECTS ON SEMEN AND HORMONAL PARAMETERS**

Mary Samplaski, MD[1], Kirk Lo, MD[2], Ethan Grober, MD[2] and Keith Jarvi, MD[2]
[1]Mount Sinai Hospital, University of Toronto, Toronto, Ontario; [2]Mount Sinai Hospital, University of Toronto
(Presented By: Mary Samplaski, MD)

Poster# 28 **ROLE OF NON-INVASIVE MARKERS IN PREDICTION OF SPERM RETRIEVAL IN NON- OBSTRUCTIVE AZOOSPERMIA**

Vasan Srini, DNB, Fellowship - Andrology and Dr. Praveen Joshi Mch
Manipal Ankur
(Presented By: Vasan Srini, DNB, Fellowship - Andrology)

Poster# 29 **INFLUENCE OF AN AROMATASE INHIBITOR ON SEXUAL FUNCTION IN MEN WITH NON-MOSAIC KLINEFELTER'S SYNDROME**

Sotirios Koukos[1], Ioannis Giakoumakis, MD[2], Evlalia Vlachopoulou, BS[1], Diamantis Daphnis, BS, PhD[2], Stavros Gratsias, MD[1], Ioannis Giannakis, MD[1], Dimitrios Baltogiannis, MD, PhD[1], Yasuyuki Mio, MD, PhD[3], Fotios Dimitriadis, MD, PhD[1], Panagiota Tsounapi, BS, PhD[4], Atsushi Takenaka, MD, PhD[4] and Nikolaos Sofikitis, MD, PhD, DMSci[1]
[1]Ioannina University School of Medicine; [2]Mediterranean Fertility Center And Genetic Services; [3]Mio Fertility Clinic; [4]Tottori University School of Medicine
(Presented By: Sotirios Koukos)

Poster# 30 **INHIBITORY PROPERTIES OF POMOGRANATE JUICE ON HUMAN CORPUS CAVERNOSUM: EXPRESSION OF NOS ISOFORMS AND PDE5A1 ENZYMES**

Serap Gur, PhD, Bashir M. Rezk, PhD, Zakaria Y. Abd Elmageed, PhD, Philip J. Kadowitz, Prof Dr, Suresh C. Sikka, Prof Dr and Wayne J.G. Hellstrom, Prof Dr
Department of Urology, Tulane University HealthSciences Center, New Orleans, Louisiana, USA
(Presented By: Serap Gur, PhD)

Poster# 31 **EVALUATION OF THE CHRONIC TREATMENT WITH RESVERATROL ON THE METABOLIC AND REPRODUCTIVE PARAMETERS OF YOUNG ADULT RATS WITH TYPE 1 DIABETES INDUCED BY STREPTOZOTOCIN IN THE PREPUBERTY**

Joana N.Simas, Postgraduate student / Master level, Vanessa V. Vilela, Collaborator and Sandra M. Miraglia, Advisor
Structural and Functional Biology Course/Department of Morphology and Genetics - Federal University of Sao Paulo - UNIFESP, Sao Paulo, Brazil
(Presented By: Joana N.Simas, Postgraduate student / Master level)

Poster# 32 **AN OBJECTIVE EVALUATION OF VIBERECT® (MALE VIBRATOR DEVICE) IN INDUCING FUNCTIONAL ERECTION IN COMPARISON TO INTRACAVERNOSAL VASOACTIVE INJECTION USING PENILE DUPLEX DOPPLER ULTRASOUND BLOOD FLOW ANALYSIS**

Suresh Sikka, PhD[1], Sree Mandava, MD[2], Khulood Kadhum[2], Nick Saragusa[2], Ronny Tan, MD[2], Kambirz Tajkarimi[2] and Wayne Hellstrom, MD, FACS[2]
[1]Tulane University School of Medicine; [2]Tulane University
(Presented By: Suresh Sikka, PhD)

Poster# 33 **SURVEY OF THE RECOGNITION OF CIRCUMCISION**

JoonYong Kim and Philip BM Kim Mr
Philip and Paul Medical Institution
(Presented By: JoonYong Kim)

Poster# 34 **IMPACT OF LIFE STYLE INTERVENTIONS ON MARKERS OF CELLULAR AGING**

Shiv Basant Kumar, MSc[1], Rashmi Yadav, MSc[2], Raj Kumar Yadav, MD[2], Madhuri Tolahunase, MSc[1], Sweta Smita Mishra, MSc[3], Kranthi Vemparala, PhD[3], Manoj Kumar, MSc[3] and Rima Dada, MD,PhD[3]
[1]Lab. for Molecular Reproduction and Genetics, Dept. of Anatomy, All India Institute of Medical Sciences, New Delhi.; [2]Integral Health Clinic(IHC), Department of Physiology, All India Institute of Medical Sciences, New Delhi; [3]Lab. for Molecular Reproduction and Genetics, Dept. of Anatomy, All India Institute of Medical Sciences, New Delhi
(Presented By: Shiv Basant Kumar, MSc)

POSTER SESSION I

Poster# 35 **EXCESSIVE EXTRACELLULAR ATP FORMATION BY MALIGNANT CELL-DERIVED PROSTASOMES DUE TO DOWNREGULATED ATPASE ACTIVITY**
K. Göran Ronquist, Anders Larsson Prof and Gunnar Ronquist Prof em
Dep. of Med. Sci
(Presented By: K. Göran Ronquist)

Poster# 36 **IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ON SUSCEPTIBILITY OF GALECTIN-3 TO CLEAVAGE BY PROSTATE SPECIFIC ANTIGEN (PSA)**
David Schoen, BS[1], Matthew Kovak, MS[1], Ashley Block, MA[1], Sarika Saraswati, PhD[2] and Alan Diekman, PhD[1]
[1]University of Arkansas for Medical Sciences; [2]Vanderbilt University
(Presented By: David Schoen, BS)

Poster# 37 **FLAGELLAR BIOGENESIS: A POTENTIAL LINK BETWEEN MFN2 AND MNS1**
Melissa Vadnais, VMD, PhD, Angel Lin, BSc, MSc and George Gerton, BSc, PhD
University of Pennsylvania
(Presented By: Melissa Vadnais, VMD, PhD)

Poster# 38 **ADENYLATE KINASE 8, ADENINE NUCLEOTIDE METABOLISM, AND A ROLE FOR AMP IN MODULATING FLAGELLAR WAVEFORMS IN MOUSE SPERM**
Melissa Vadnais, VMD, PhD, Wenlei Cao, BMS, PhD[1], Haig Aghajanian, BSc[2], Lisa Haig-Ladewig, BSc[2], Angel Lin, BSc, MSc[2] and George Gerton, BSc, PhD[2]
[1]University of Massachusetts; [2]University of Pennsylvania
(Presented By: Melissa Vadnais, VMD, PhD)

Poster# 39 **VARICOCELECTOMY: CLINICAL IMPLICATIONS AND PROGNOSIS IN MANAGEMENT OF INFERTILITY.**
Monis Bilal Shamsi, MSc, PhD and Rima Dada, MD, PhD
Laboratory for Molecular Reproduction and Genetics, All India Institute of Medical Science
(Presented By: Monis Bilal Shamsi MSc,PhD)

Poster# 40 **THE CATSPER CALCIUM CHANNEL IN HUMAN SPERMATOZOA: RELATION WITH MOTILITY AND INVOLVEMENT IN PROGESTERONE-INDUCED ACROSOME REACTION**
Lara Tamburrino, PhD, Sara Marchiani, PhD, Gianni Forti, MD, Monica Muratori, PhD and Elisabetta Baldi, PhD
University of Florence
(Presented By: Lara Tamburrino, PhD)

Poster# 41 **SPERM'S MEMBRANE CHARGE: AN INTERESTING BIOMARKER FOR NON-INVASIVE METHOD OF SPERM SELECTION**
Luke Simon, PhD and Douglas Carrell, PhD, HCLD
University of Utah
(Presented By: Luke Simon, PhD)

Poster# 42 **HUMAN BINDER OF SPERM PROTEIN HOMOLOG 1 (BSPH1) CAN PROMOTE SPERM CAPACITATION**
Genevieve Plante, Isabelle Therien, PhD[1], Catherine Lachance, PhD[2], Pierre Leclerc, PhD[3] and Puttaswamy Manjunath, PhD[4]
[1]HMR Research Center; [2]Unversité Laval; [3]Université Laval; [4]Université de Montréal
(Presented By: Genevieve Plante)

Poster# 43 **QUANTITATIVE PHOSPHOPROTEOMIC ANALYSIS OF SPERM CAPACITATION REVEALS A KEY ROLE OF IGF1R TYROSINE KINASE IN HUMAN**
Jing Wang, PhD, Candidate[1], Lin Qi, PhD, Candidate[2], Tao Zhou, PhD, Candidate[2], Yueshuai Guo, PhD, Candidate[2], Gaigai Wang, Master[2], Zuomin Zhou, PhD[2], Xuejiang Guo, PhD[2] and Jiahao Sha, PhD[2]
[1]State Key Laboratory of Reproductive Medicine,Nanjing Medical University,Nanjing 210029,China; [2]State Key Laboratory of Reproductive Medicine,Nanjing Medical University,Nanjing 210029,China
(Presented By: Jing Wang, PhD, Candidate)

Poster# 44 **INSIGHTS INTO THE LYSINE ACETYLATION OF PROTEINS IN CAPACITATED HUMAN SPERM**
Xuejiang Guo, PhD[1], Guohai Sun, Master Candidate[2], Min Jiang, PhD Candidate[2], Tao Zhou, PhD, Candidate[2], Yiqiang Cui, Master Candidate[2], Yueshuai Guo, PhD Candidate[2], Zuomin Zhou, PhD[2] and Jiahao Sha, PhD[2]
[1]State Key Laboratory of Reproductive Medicine, Nanjing Medical University; [2]State Key Laboratory of Reproductive Medicine, Department of Histology and Embryology, Nanjing Medical University, Nanjing 210029, China
(Presented By: Xuejiang Guo, PhD)

POSTER SESSION I

Poster# 45 **SPERM MOTILITY LOSS AND ACTIVATION OF THE CAMP-PKA PATHWAY CAUSED BY THE STAT3 INHIBITORY COMPOUND V RESULT FROM EXCESSIVE REACTIVE OXYGEN SPECIES PRODUCTION.**
Catherine Lachance, PhD, Serge Goupil, BSc, Roland R. Tremblay, DSc, MD, PhD and Pierre Leclerc, PhD
Université Laval
(Presented By: Catherine Lachance, PhD)

Poster# 46 **ROBUST AUTOMATIC SPERM TRACKING**
Leonardo Urbano, MSEE[1], Matthew D. VerMilyea, PhD[2], Puneet Masson, MD[2] and Moshe Kam, PhD[1]
[1]Drexel University; [2]Penn Fertility Care
(Presented By: Leonardo Urbano, MSEE)

Poster# 47 **JUSTICIA GANDARUSSA BURM.F. AS HYALURONIDASE HUMAN SPERMATOZOA INHIBITOR ACTIVITY**
Bambang Prajogo
(Presented By: Bambang Prajogo)

Poster# 48 **REGULATION OF ACROSOME REACTION BY LIPRIN ?3, LAR AND ITS LIGANDS IN MOUSE SPERM**
Chetanchandra Joshi, Msc, Shagufta Khan, PhD and Vrinda Khole, PhD
National Institute for Research in Reproductive Health
(Presented By: Chetanchandra Joshi Msc)

Poster# 49 **ASSESSMENT OF SPERM DNA FRAGMENTATION AFTER MICROSCOPIC SUBINGUINAL VARICOCELECTOMY INTRODUCTION:**
Brooke Harnisch, MD and Jay Sandlow, MD
Medical College of Wisconsin
(Presented By: Brooke Harnisch, MD)

Poster# 50 **THE RELATIONSHIP BETWEEN SPERM VIABILITY AND DNA FRAGMENTATION RATES**
Mary Samplaski, MD[1], Apostolos Dimitromanolakis, MSc[2], Brendan Mullen, MD[2], Kirk Lo, MD[2], Ethan Grober, MD[2] and Keith Jarvi, MD[2]
[1]Mount Sinai Hospital, University of Toronto, Toronto, Ontario; [2]Mount Sinai Hospital, University of Toronto
(Presented By: Mary Samplaski, MD)

Poster# 51 **CHARACTERIZATION OF MEMBRANE OCCUPATION AND RECOGNITION NEXUS REPEAT CONTAINING 3, A MEIG1 BINDING PARTNER, IN MOUSE MALE GERM CELLS**
Ling Zhang, Hongfei Li, MD, Yuqin Shi, PhD, Maria Teves, PhD, Zhiqiong Wang, MD, Gaofeng Jiang, PhD, Shizhen Song, PhD and Zhibing Zhang, PhD
(Presented By: Ling Zhang)

Poster# 52 **A MEIG1/PACRG COMPLEX IN THE MANCHETTE IS ESSENTIAL FOR THE TRANSPORT OF STRUCTURAL PROTEINS REQUIRED FOR CONSTRUCTION OF THE SPERM FLAGELLA**
Maria Teves, David Nagarkatti-Gude, Kellie Archer, Waixin Tang, Darrell Peterson, Jerome Strauss and Zhibing Zhang
(Presented By: Zhibing Zhang)

Poster# 53 **COMBINED ADMINISTRATION OF CURCUMIN AND GALLIC ACID INHIBITS GALLIC ACID-INDUCED SUPPRESSION OF STEROIDOGENESIS, SPERM OUTPUT, ANTIOXIDANT DEFENSES AND INFLAMMATORY RESPONSIVE GENES**
Sunny Abarikwu, PhD, Mojisola Durojaiye, BSc, Adenike Alabi, BSc and Oghenetega Akiri, BSc
Redeemer's University, Nigeria
(Presented By: Sunny Abarikwu, PhD)

Poster# 54 **THE TRANSCRIPTION FACTOR MEF2 IS A NOVEL REGULATOR OF GSTA1 EXPRESSION IN MA-10 LEYDIG CELLS**
Mickael Di-Luoffo, MSc, Catherine Brousseau, MSc, Francis Bergeron, MSc and Jacques J. Tremblay, PhD
CRCHUQ-Universite Laval
(Presented By: Mickael Di-Luoffo, MSc)

Poster# 55 **DETECTION OF STRONGLY REPRESSED AND HIGHLY ACTIVE MRNAS IN THE CHROMATOID BODY OF ROUND SPERMATIDS WITH A SIMPLE AND SENSITIVE FLUORESCENT IN SITU HYBRIDIZATION TECHNIQUE**
Danielle Cullinane, Graduate Student and Ken Kleene, PhD
Umass Boston
(Presented By: Danielle Cullinane, Graduate Student)

POSTER SESSION I

Poster# 56 **A-SINGLE SPERMATOGONIA HETEROGENEITY AND CELL CYCLE SYNCHRONIZE WITH A SPECIFIC RAT SEMINIFEROUS EPITHELIAL STAGE**
Shadaan N. Abid, PhD, Timothy E. Richardson, MD, PhD, Heather M. Powell, MS, Priscilla Jaichander, PhD, Jaideep Chaudhary, BS, Karen M. Chapman, BS and F. Kent Hamra, PhD
UT Southwestern Medical Center in Dallas
(Presented By: F. Kent Hamra, PhD)

Poster# 57 **LINKING SPERMATID RNA BINDING PROTEIN DIVERSITY TO REPRODUCTIVE SUCCESS**
Karen M. Chapman, BS, Jaideep Chaudhary, BS, Timothy E. Richardson, MD, PhD and F. Kent Hamra, PhD
UT Southwestern Medical Center in Dallas
(Presented By: F. Kent Hamra, PhD)

Poster# 58 **EFFECTS OF ALLII TUBEROSI SEMEN ON THE CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) EXPRESSION DURING SPERMATOGENESIS**
Jin hyoung Cho, MS, Sung Won Jee, PhD, Do Rim Kim, PhD, Ha Young Kim, MS, Eun Bit Ko, MS, Ho Jin Lee, MS, Mun Seog Chang, PhD and Seong Kyu Park, PhD
Department of Prescriptionology, College of Korean Medicine, Kyung Hee University
(Presented By: Seong Kyu Park, PhD)

Poster# 59 **PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-?/D (PPAR?/D) REGULATES SPERMATOGENESIS BY ALTERING CELL-CYCLE REGULATORS IN MICE**
Pei-Li Yao, LiPing Chen, Frank Gonzalez and Jeffrey Peters
(Presented By: Pei-Li Yao)

Poster# 60 **EFFECT OF IRRADIATION ON THE LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MOUSE TESTIS**
Mahmoud Huleihel, PhD, Tal Dadon, BSc, Jenny Rechkin, BSc and Eitan Lunenfeld, MD
Ben-Gurion University of the Negev
(Presented By: Mahmoud Huleihel, PhD)

Poster# 61 **INHIBITION OF MTOR SIGNALING DECREASES STRA8 EXPRESSION IN ADULT MOUSE TESTIS**
Pinar Sahin, MSc[1], Zeliha Sahin, PhD[2], N. Ece Gungor-Ordueri, MSc[1] and Ciler Celik-Ozenci, DDS, PhD[1]
[1]Akdeniz University Medical Faculty Department of Histology and Embryology; [2]Near East University Medical Faculty Department of Histology and Embryology
(Presented By: Pinar Sahin, MSc)

Poster# 62 **FUNCTIONAL CHARACTERIZATION OF ION CHANNELS IN SINGLE SPERMATOGONIA IN VITRO AND IN SITU**
David Fleck, Master of Science (MSc)[1], Sophie Veitinger, PhD[2], Thomas Veitinger, PhD[1], Patricia Almeida Machado, BSc[1], Susanne Lipartowski[1], Corinna Engelhardt[1], Jennifer Spehr, PhD[1] and Marc Spehr, PhD[1]
[1]Department of Chemosensation, Institute for Biology II; [2]Institute for Cytobiology, Philipps-University Marburg
(Presented By: David Fleck, Master of Science (MSc))

Poster# 63 **FURTHER CONFIRMATION OF SEVERAL IMPORTANT TARGETS OF SUMOYLATION IN TESTICULAR CELLS**
Yuxuan Xiao, PhD[1], Daniel Pollack, BsC[1], Avi Levy[1], Miriam Andrusier[1] and Margarita Vigodner, PhD[2]
[1]Yeshiva University; [2]Yeshiva University and AECOM, New York.
(Presented By: Margarita Vigodner, PhD)

Poster# 126 **RESPONSIVENESS OF THE SPERMATOGONIAL STEM CELL POOL TO RETINOIC ACID**
Ryan Anderson, BS, Melissa Oatley, MS and Jon Oatley, PhD
Washington State University
(Presented By: Ryan Anderson, BS)

*POSTER SESSION II

**Not CME Accredited*
Monday, April 7, 2014
11:15 a.m. – 12:30 p.m.
Location: Venetian

Poster# 64 **THE TRANSCRIPTION FACTOR SOX9 IS A NOVEL REGULATOR OF STEROIDOGENIC GENES EXPRESSION IN MA-10 LEYDIG CELLS**
David Landry, BSc and Luc J. Martin, PhD
Université de Moncton
(Presented By: David Landry, BSc)

Poster# 65 **EFFECTS OF METHOXYCHLOR AND ITS METABOLITE 2,2-BIS(P-HYDROXYPHENYL)-1,1,1- TRICHLOROETHANE ON HUMAN AND RAT**
Leping Ye, MD[1], Ren-Shan Ge, PhD[1] and Hui Li, MD, PhD[2]
[1]The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University; [2]Division of Neonatology, the First Affiliated Hospital of School of Medicine, Xi'an Jiaotong University
(Presented By: Leping Ye, MD)

Poster# 66 **EXPRESSIONS OF SOX5 AND SOX13 TRANSCRIPTION FACTORS ARE INCREASED IN TESTICULAR LEYDIG CELLS OF RODENTS DURING POST-NATAL DEVELOPMENT**
Mikella A. Daigle, BSc and Luc J. Martin, PhD
Université de Moncton
(Presented By: Mikella A. Daigle, BSc)

Poster# 67 **DEHYDROEPIANDROSTERONE ANTAGONIZES SURGERY STRESS-INDUCED SUPPRESSION OF TESTOSTERONE PRODUCTION IN MALE RATS**
Han Lin, PhD, MD[1], Kaimin Yuan, PhD, MD[2], Hongyu Zhou, PhD[2], Dongxin Chen, PhD[2], Tiao Bu Master[2], Shiwen Liu, Master[2], Jingyang Li, PhD[2], Qingquan Lian, PhD, MD[2] and Renshan Ren, PhD, MD[2]
[1]Wenzhou Medical University.The Second Hospital,; [2]Wenzhou Medical University, The Second Hospital
(Presented By: Han Lin, PhD, MD)

Poster# 68 **IMPROVING THE FERTILITY OF DOG**
Gamal El-Amrawi, Professor
Alexandria University
(Presented By: Gamal El-Amrawi, Professor)

Poster# 69 **PREVALENCE OF BONE DENSITY DEFICIENCIES IN MEN PRESENTING FOR HYPOGONADISM TREATMENT: DO WE NEED TO WORRY?**
Igor Sorokin, MD[1], Paul Feustel, PhD[2] and Andrew McCullough, MD[3]
[1]Albany Medical College; [2]albany medical college; [3]Urological Institute of North Eastern New York
(Presented By: Igor Sorokin, MD)

Poster# 70 **CHRONIC CYCLOPHOSPHAMIDE TREATMENT AFFECTS GENE EXPRESSION IN PACHYTENE SPERMATOCYTES AND ROUND SPERMATIDS**
Anne Marie Downey, Barbara Hales, PhD and Bernard Robaire, PhD
McGill University
(Presented By: Anne Marie Downey)

Poster# 71 **ACTION OF RESVERATROL ON THE REPRODUCTIVE PARAMETERS OF LATE PUBERTAL RATS TREATED WITH ANTI-CANCER AGENTS (BEP PROTOCOL MODIFIED), FROM PERIPUBERTY**
Flavia Macedo de Oliveira Neves, PhD student, Vanessa Vendramini Vilela, Collaborator and Sandra Maria Miraglia, Advisor
Federal University of Sao Paulo - UNIFESP - Brazil
(Presented By: Flavia Macedo de Oliveira Neves, PhD student)

Poster# 72 **FETAL CYCLOPHOSPHAMIDE EXPOSURE INDUCES TESTICULAR CANCER AND REDUCES SPERMATOGENESIS IN MICE**
Gunapala Shetty, PhD, Ana Luiza Drumond, PhD, Paul Comish, MS, Angabin Matin, PhD and Marvin Meistrich, PhD
University of Texas M.D. Anderson Cancer Center
(Presented By: Gunapala Shetty, PhD)

POSTER SESSION II

Poster# 73 **EFFECTS OF EUCOMMIAE CORTEX (EC) ON SPERM COUNT AND MOTILITY PARAMETERS IN MALE MICE**

Ji Eun Lee, MS, Eun Bit Ko, MS, Jin Soo Kim, PhD, Do Rim Kim, PhD, Ha Young Kim, MS, Byung Chun Park, MS, Bong Jae Choi, PhD, Seong Kyu Park, PhD and Mun Seog Chang, PhD
Department of Prescriptionology, College of Korean Medicine, Kyung Hee University
(Presented By: Mun Seog Chang, PhD)

Poster# 74 **LATE REPRODUCTIVE ANALYSIS OF RAT MALE OFFSPRING EXPOSED TO NICOTINE DURING PREGNANCY AND LACTATION – FINAL PART**

Mayra Miranda-Rodrigues, Masters Student, Camila C. Paccola, Collaborator, Samara U. Oliva, Collaborator and Sandra M. Miraglia, Advisor
Federal University of Sao Paulo - UNIFESP - Brazil
(Presented By: Mayra Miranda-Rodrigues, Masters Student)

Poster# 75 **MOLECULAR ALTERATIONS IN SPERM ARE SENSITIVE INDICATORS OF TESTICULAR DYSFUNCTION**

Linnea Anderson, MSc[1], Edward Dere, PhD[2], Susan Huse, PhD[1] and Kim Boekelheide, MD, PhD[1]
[1]Brown University; [2]Rhode Island Hospital
(Presented By: Linnea Anderson, MSc)

Poster# 76 **NETWORK ANALYSIS OF REDOX MEDIATED PROTEIN-PROTEIN INTERACTIONS IN SPERMATOZOA**

Burak Özkösem
McGill University
(Presented By: Burak Özkösem)

Poster# 77 **MAPPING THE SPERM MEMBRANE PROTEIN INTERACTOME**

Matthew Marcello, PhD[1], Marina Druzhinina[2] and Andrew Singson, PhD[2]
[1]Pace University; [2]Waksman Institute - Rutgers University
(Presented By: Matthew Marcello, PhD)

Poster# 78 **COMPARATIVE ANALYSIS OF MACAQUE AND HUMAN SPERM PROTEOMES: INSIGHTS INTO SPERM COMPETITION**

Tao Zhou[1], Gaigai Wang[1], Minjian Chen[2], Yueshuai Guo[1], Zuomin Zhou[1], Jiahao Sha[1] and Xuejiang Guo[1]
[1]State Key Laboratory of Reproductive Medicine, Department of Histology and Embryology, Nanjing Medical University; [2]Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University
(Presented By: Tao Zhou)

Poster# 79 **TRANSCRIPTIONAL PROFILING OF HYPOXIA PATHWAY GENE EXPRESSION IN THE RAT TESTIS FOLLOWING P. AERUGINOSA LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION**

Genevieve Fasano[1] and Michael Palladino, PhD[2]
[1]Biology Department, Monmouth University; [2]Monmouth University
(Presented By: Genevieve Fasano)

Poster# 80 **OLIGOZOOSPERMIA TRANSCRIPTOME PROFILE AND GENE CANDIDATE DISCOVERY IN SEMEN FROM INFERTILE MALES**

Andrew P. Georgiadis, BA[1], Archana Kishore, PhD[1], Tamanna Sultana, PhD[2], James Lyons-Weiler, PhD[2], Etta Volk, MS[3], Thomas M. Jaffe, MD[3], Joseph S. Sanfilippo, MD[3], Aleksandar Rajkovic, MD, PhD[1] and Alexander N. Yatsenko, MD, PhD[1]
[1]MWRI; [2]University of Pittsburgh; [3]MWH
(Presented By: Alexander N. Yatsenko, MD, PhD)

Poster# 81 **WHEREAS ALL CASES OF FAILED FERTILIZATION WITH CONVENTIONAL OOCYTE INSEMINATION WITH NORMAL SPERM ACHIEVE GOOD FERTILIZATION RATES WITH ICSI ONLY HALF WITH NORMAL BINDING HAVE GOOD FERTILIZATION RATES**

Jerome Check, MD, PhD[1], Aniela Bollendorf, MT, HEW[2] and Carrie Wilson, BA[3]
[1]Medical School at Rowan University; [2]Cooper Institute for Reproductive Hormonal Disorders; [3]Cooper Institute for Reproductive and Hormonal Disorders
(Presented By: Jerome Check, MD, PhD)

Poster# 82 **SPERM WITH LOW HYPO-OSMOTIC SWELLING (HOS) TEST SCORES MAY BE A RARE CAUSE OF RECURRENT MISCARRIAGE**

Jerome Check, MD, PhD[1] and Aniela Bollendorf, MT, HEW[2]
[1]Medical School at Rowan University; [2]Cooper Institute for Reproductive Hormonal Disorders
(Presented By: Jerome Check, MD, PhD)

POSTER SESSION II

Poster# 83 **THE ROLE OF JUSTICIA GENDARUSSA BURM.F., AS MALE CONTRACEPTION, ON BLOOD LIPID PROFILE**

Sri Musta'ina, Master[1], Bambang Prajogo, Proffessor[2], Bayu Al Huda, Trainee[2] and Dyan Pramesti, MD, Master[3]
[1]RHRC Dept Biomedical Science School of medicine Airlangga University; [2]Faculty of Pharmacy Airlangga University; [3]Reproductive Health Research Centre, Dept Biological Science School of Medicine Airlangga University
(Presented By: Sri Musta'ina, Master)

Poster# 84 **INVESTIGATING THE SPERMICIDAL PROPERTIES OF NOVEL COMPOUNDS**

Ashley Robertson and Jennifer Venditti, PhD, MS, BS
Bloomsburg University
(Presented By: Ashley Robertson)

Poster# 85 **A MODEL FOR STUDYING PROTECTION FROM INFERTILITY AFTER CHEMOTHERAPY: CYCLOPHOSPHAMIDE (CYP) DECREASES METASTATIC LUNG MELANOMA FORMATION AND INCREASES GERM CELL APOPTOSIS IN MICE**

YanHe Lue, MD[1], Ronald Swerdloff, MD[1], Kyeong-Ri Yu[1], Kevin Bruhn, PhD[2] and Christina Wang, MD[1]
[1]Division of Endocrinology, Department of Medicine, Los Angeles Biomedical Research Institute and Harbor-UCLA Medical Center; [2]Division of Dermatology
(Presented By: YanHe Lue, MD)

Poster# 86 **DEVELOPMENT OF MALE NON-HORMONAL CONTRACEPTIVES BY TARGETING LATE SPERMIOGENESIS**

Shuiqiao Yuan, PhD, Zong-Liang Chang, MSc, Huili Zheng, MD, PhD and Wei Yan, MD, PhD
University of Nevada School of Medicine
(Presented By: Wei Yan, MD, PhD)

Poster# 87 **HIDE AND SEEK WITH SPERMS:MICROTESE AN OPTION IN NON OBSTRUCTIVE AZOOSPERMIA**

Dharmaraj Palanisamy, DNB (UROLOGY), Sandro C. Esteves, MD (ANDROLOGIST), Mohammed Ashraf, MD (IVF SPECIALIST), Sankalp Singh, MD (IVF SPECIALIST) and Sujatha Ramakrishnan, PhD (Embryologist)
(Presented By: Dharmaraj Palanisamy, DNB (UROLOGY))

Poster# 88 **ABSORBABLE CYANOACRYLATE FOR USE IN MICROSURGICAL VASOVASOTOMY:A NOVEL METHOD TO REINFORCE THE ANASTOMOSIS**

Tariq S. Hakky, MS, Alberto,J. Duboy, MD[1], Larry I. Lipshultz, MD[2] and Rafael E. Carrion, MD[1]
[1]USF; [2]Baylor College of Medicine
(Presented By: Tariq S. Hakky, MS)

Poster# 89 **ABSENCE OF NHERF-2 IN EPIDIDYMIS INCREASES LUMINAL SIZE THROUGH DYSREGULATION OF VATPASE LOCALIZATION.**

Jose Mira, MPH[1], Wayland Hsiao, MD and Mitsi Blount, PhD[2]
[1]Morehouse School of Medicine; [2]Emory University
(Presented By: Wayland Hsiao, MD)

Poster# 90 **INCIDENCE OF MALE AND FEMALE STERILIZATION FOLLOWING A RECENT LIVE BIRTH:ESTIMATES FROM THE PREGNANCY RISK ASSESSMENT MONITORING SYSTEM (PRAMS), 2007-2010**

Lee Warner, PhD[1], Mary Elizabeth O'Neil, MPH[1], Lorrie Gavin, PhD[1], Letitia Williams, MPH[1], Shanna Cox, MSPH[1], Michael Eisenberg, MD[2], Jim Hotaling, MD[3] and Denise Jamieson, MD[1]
[1]CDC; [2]Stanford University; [3]University of Utah
(Presented By: Lee Warner, PhD)

Poster# 91 **PIOGLITAZONE INCREASES CYCLIC GMP CONCENTRATIONS IN A RAT MODEL OF POST-PROSTATECTOMY ERECTILE DYSFUNCTION**

Louis Aliperti[1], Sharika Hagan[2], George Lasker[2], Joshua Hellstrom[2], Landon Trost, MD[3], Suresh Sikka, PhD[2] and Wayne Hellstrom, MD, FACS[2]
[1]Tulane University School of Medicine; [2]Tulane University; [3]Mayo Clinic
(Presented By: Louis Aliperti)

POSTER SESSION II

Poster# 92 **THE PENILE DOPPLER PARAMETERS AND CLINICAL RISK FACTORS IN MEN WITH ERECTION HARDNESS SCORE 3-4 AFTER INTRACAVERNOUS INJECTION**

Qiang Li, Reena Kabaria, MD, Carolyn Cutler, Andrew Ostrowski, Zachary Klaassen, MD, Patrick Fox, MD, Casey McCraw, MD, Roger Chen, MD, Brittani Barrett and Ronald Lewis, MD
(Presented By: Qiang Li)

Poster# 93 **SEXUAL FUNCTION IN MALE PARTNERS OF WOMEN PARTICIPATING IN A SURROGATE MOTHERHOOD PROGRAM**

Ioannis Giakoumakis, MD, Diamantis Daphnis, BS, PhD[1], Evlalia Vlachopoulou, BS, PhD[2], Konstantinos Kotsonas, MD[2], Nikolaos Kolitsas, MD[2], Stavros Gratsias, MD[2], Panagiota Tsounapi, BS, PhD[3], Sotirios Kouko,s MD, FEBU[2], Ioannis Giannakis, MD[2], Atsushi Takenaka, MD,PhD[3] and Nikolaos Sofikitis, MD, PhD, DMSci[2]
[1]Mediterranean Fertility Institute; [2]Ioannina University; [3]Tottori University
(Presented By: Ioannis Giakoumakis MD)

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Panagiota Tsounapi, PhD[1], Motoaki Saito, MD, PhD[2], Fotios Dimitriadis, MD, PhD, FEBU[3], Shogo Shimizu, MSc, PhD[4], Masashi Honda, MD, PhD[1], Michiyo Iguchi, BS[4], Shinji Matsunaga, MSc, PhD[4], Kuniyasu Muraoka, MD, PhD[1], Takehiro Sejima, MD, PhD[1], Shuhei Tomita, MD, PhD[4], Nikolaos Sofikitis, MD, PhD, DMSci[3] and Atsushi Takenaka, MD, PhD[1]
[1]Division of Urology, Tottori University School of Medicine; [2]Department of Pharmacology, Kochi University School of Medicine; [3]Department of Urology, University of Ioannina School of Medicine; [4]Division of Molecular Pharmacology, Tottori University School of Medicine
(Presented By: Panagiota Tsounapi, PhD)

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Libor Zamecnik, MD, PhD,[1], Tatana Sramkova, MD, PhD[2], Katerina Sramkova, MD[3] and Tomas Hradec, MD[4]
[1]Dpt. of Urology, 1st Medical School, Charles University, Prague; [2]Department of Urology, 1st Faculty of Medicine and General University, Department of Sexology, University Hospital, Brno, Czech Republic; [3]Department of Urology, St Annes Hospital, Brno, Czech Republic; [4]Dpt. of Urology, 1st Medical School, Charles University, Prague, Czech Republic
(Presented By: Libor Zamecnik, MD, PhD,)

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Amy Lee Chong, MD[1], Keith Jarvi, MD[2], Kirk Lo, MD[2], Sergey Moskovtsev, MD, PhD[3], Brendan Mullen, MD[4], Leia Spencer, RN[2], David Hodgson, MD[5], Mark Greenberg, MD[1] and Paul Nathan, MD[1]
[1]Department of Hematology/Oncology, The Hospital for Sick Children, and Paediatric Oncology Group of Ontario, Toronto, Canada; [2]Murray Koffler Urologic Wellness Centre, Mount Sinai Hospital, Toronto, Ontario, Canada; [3]CReATe Fertility Centre and Department of OBGYN, University of Toronto,Toronto, Canada; [4]Department of PML, Mount Sinai Hospital, Toronto, Ontario, Canada; [5]Department of Radiation Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada
(Presented By: Sergey Moskovtsev, MD, PhD)

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CDC
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Xiao Gu, MD, PhD[1], Motoo Araki, MD, PhD[2] and Wong Carson, MD[3,4,5]
[1]First Clinical Medical College at Yangzhou University, Yangzhou; [2]Okayama University Graduate School of Medicine; [3]SouthWest Urology, LLC; [4]Parma Community General Hospital; [5]University Hospitals Ahuja Medical Center
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Aniela Bollendorf, MT, HEW[1] and Jerome Check, MD, PhD[2]
[1]Cooper Institute for Reproductive Hormonal Disorders; [2]Cooper Medical School at Rowan University
(Presented By: Aniela Bollendorf MT, HEW)

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Sharika Hagan[1], Debasis Mondal, PhD[2], Asim Abdel-Mageed, PhD[2], Wayne Hellstrom, MD, FACS[2] and Suresh Sikka, PhD[2]
[1]Tulane University School of Medicine; [2]Tulane University
(Presented By: Sharika Hagan)

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Amin Herati, MD[1], Andrew De Jesus, BS[2], Gideon Richards, MD[3] and Bruce Gilbert, MD, PhD[4]
[1]The Smith Institute for Urology, Hofstra University-North shore/Long Island Jewish Health System; [2]The Men's Fertility Laboratory and New York Cryo; [3]The Smith Institute for Urology, Hofstra University-North Shore/Long Island Jewish Health System.; [4]The Smith Institute for Urology, Hofstra University-North Shore/Long Island Jewish Health System. & The Men's Fertility Laboratory and New York Cryo
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Erik Yao, BSc, Mary Samplaski, MD, Kirk Lo, MD, Ethan Grober, MD and Keith Jarvi, MD
Mount Sinai Hospital, University of Toronto, Toronto, Ontario
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Fertility Solutions
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Fertility Solutions
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Gayathri Devi Rajamanickam, John Kastelic, DVM, PhD, Dip ACT[1] and Jacob Thundathil, DVM, MVSc, PhD[2]
[1]Professor and Head, Dept. of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary; [2]Associate Professor, Dept. of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary
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Cibele Melo, Doctorate Student[1], Cecilia Lima, Undergraduate Student[2], Rebeca Ramos, Undergraduate Student[3] and Erika Oliveira, PhD[4]
[1]Northeastern Biotechnology Network (RENORBIO), Recife, PE, Brazil; [2]UFRPE, Department of Veterinary Medicine, Recife, PE, Brazil; [3]UFRPE, Department of Veterináry Medicine, Recife, PE, Brazil; [4]UFRPE
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Anthony Laroche, BScA Agr[1], Alexandre Bastien, MSc, Phy[1], Christine Guillemette, MSc[1], Patrick Vincent, PhD[2], Patrick Blondin, PhD[2] and François Richard, PhD[1]
[1]Laval University; [2]L'Alliance Boviteq Inc.
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[1]Fertility Solutions; [2]National Institute for Occupational Safety and Health
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Jannette Dufour, PhD, Gurvinder Kaur, PhD and Nicky Paniagua
Texas Tech University Health Sciences Center
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Vasan Srini, DNB, Fellowship - Andrology[1], Dr. Praveen Joshi, Mch[1], Darshan SC, PhD[2] and Acharya KK, PhD[2]
[1]Manipal Ankur; [2]Institute of Bioinformatics & Applied Biotechnology - IBAB
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Romain Lambrot, PhD[1], Christine Lafleur, MSc[1], Michael G. Rosenfeld, MD[2] and Sarah Kimmins, PhD[3]
[1]Department of Animal Science McGill University, Canada; [2]Howard Hughes Medical Institute, University of California at San Diego School of Medicine, USA; [3]Department of Animal Science and Department of Pharmacology and Therapeutics, McGill University, Canada
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Lee Smith, BSc, PhD
MRC Centre for Reproductive Health
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Qi-En Yang, PhD and Jon M. Oatley, PhD
Washington State University
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[1]Department of Biochemistry & Molecular Biology, Monash University, Clayton, VIC, Australia; [2]Priority Research Centre in Reproductive Science, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW, Australia; [3]Department of Anatomy & Developmental Biology, Monash University, Clayton, VIC, Australia; [4]Departments of Biochemistry & Molecular Biology and Anatomy & Cell Biology, Monash University, Clayton, VIC, Australia
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Nilam Sinha, Luis Korrodi-Gregorio, PhD[1], Douglas Kline, PhD[2] and Srinivasan Vijayaraghavan, PhD[2]
[1]University of Aveiro, Aveiro, Portugal; [2]Kent State University, Kent, OH
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Andrew Midzak, PhD[1], Nagaraju Akula, PhD[2] and Vassilios Papadopoulos, PhD, PharmD[3]
[1]McGill University; [2]Research Institute of the McGill University Health Centre; [3]McGill University, Research Institute of the McGill University Health Centre
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Matthew Wosnitzer, MD[1], Anna Mielnik, MS[1], Ali Dabaja, MD[1], Brian Robinson, MD[2], Peter Schlegel, MD[1] and Darius Paduch, MD[1]
[1]Department of Urology, Weill Cornell Medical College, New York, NY 10065; [2]Department of Pathology, Weill Cornell Medical College, New York, NY 10065
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Leeyah Issop, PhD, Jinjnag Fan, PhD, Sunghoon Lee, MSC, Malena B. Rone, PhD and Vassilios Papadopoulos, DPharm, PhD
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Evlalia Vlachopoulou, BS, PhD[1], Ioannis Giakoumakis, MD[2], Diamamtis Daphnis, BS, PhD[2], Ioannis Georgiou, BS,PhD[3], Dimitrios Baltogiannis, MD, PhD4, Leandros Lazaros, BS,PhD[3], Ioannis Giannakis, MD[4], Panagiota Tsounapi, BS,PhD[5], Atsushi Takenaka, MD, PhD[5] and Nikolaos Sofikitis, MD, PhD, DMSci[4]
[1]Post Doctoral Position; [2]Mediterranean Fertility Center And Genetic Services; [3]Ioannina University, Obstetrics and Gynecology; [4]Ioannina University, Department of Urology; [5]Tottori University, Department of Urology
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Sunday, April 6, 2014
2:00 p.m. - 3:30 p.m.

Concurrent Oral Session I: Molecular and Environmental Regulation of Male Reproductive Health
Location: Windsor C - E
Session Chairs:
Kate Loveland, PhD and Jacquetta M. Trasler, MD, PhD

1

RESPONSIVENESS OF THE SPERMATOGONIAL STEM CELL POOL TO RETINOIC ACID

Ryan Anderson, BS, Melissa Oatley, MS and Jon Oatley, PhD
Washington State University
(Presented By: Ryan Anderson, BS)

Introduction: Continual spermatogenesis relies on the actions of an undifferentiated spermatogonial population comprised of progenitor and stem cell (SSC) subtypes. Self−renewal maintains a foundational pool of SSCs from which progenitor spermatogonia arise and transiently amplify in number before committing to a pathway of terminal differentiation. Transition to a differentiating state is controlled by retinoic acid (RA) signaling and a hallmark is attained expression of the Kit receptor. Response of SSCs to RA signaling in a similar manner to that occurring in progenitors would lead to loss of the SSC pool and subsequent fertility defects and therefore must be suppressed. At present, it is unknown whether SSCs possess the capacity for response to RA.
Methods: To address this, we utilized primary cultures of undifferentiated spermatogonia established from a transgenic mouse model in which the SSC fraction is labeled by GFP expression thereby allowing for separation of SSC and progenitor spermatogonial subtypes.
Results: Results of RT−PCR analyses demonstrated that transcripts for retinoic acid receptor gamma (RARg) and retinoid x receptors alpha (RXRa) and beta (RXRb) are present in both SSC and progenitor spermatogonia. This finding was confirmed by immunofluorescent staining of testis cross−sections. Next, we investigated whether RA induced transition from a Kit− to Kit+ state is different between SSC and progenitor spermatogonia. Using flow cytometric analysis we found that ~38% of cells within the progenitor spermatogonial fraction were Kit+ after overnight exposure to RA. In contrast, only ~15% of cells in the SSC fraction were Kit+ after identical treatment. Without RA exposure >1% of cells were Kit+ in both fractions. Lastly, utilizing transplantation analyses we found that the number of cells capable of regenerating spermatogenesis was not different in cultures subjected to overnight RA exposure compared to controls.
Conculsion: Collectively, these findings indicate that both SSC and progenitor spermatogonia subtypes possess the molecular machinery for induction of RA signaling but the response is dramatically different with SSCs retaining regenerative capacity while progenitor spermatogonia transition to a differentiating state. Disruption in the ability for SSCs to remain unresponsive to the differentiating influence of RA signaling could be an underlying cause of male infertility. This research was supported by grant HD061665 from the National Institutes of Health.

2

THE TRANSLATIONAL REPRESSOR, Y−BOX PROTEIN 2 (YBX2/MSY2), BINDS THE CIS−ELEMENT (TCE) THAT INACTIVATES MOUSE PRM1 MRNA TRANSLATION IN ROUND SPERMATIDS.

Tamjid Chowdhury, BA and Kenneth Kleene, PhD
University of Massachusetts Boston
(Presented By: Kenneth Kleene, PhD)

Introduction and Objectives: The protamine (Prm) and transition protein (Tnp) mRNAs exemplify a wide−spread pattern of developmental regulation of mRNA translation in which mRNAs are transcribed in early spermatids, stored as translationally inactive free−mRNPs and actively translated on polysomes in transcriptionally inert late spermatids. Studies of mutations in transgenic mice define a translation control element (TCE) in the Prm1 3'UTR that is necessary and sufficient for translational repression in early spermatids (Zhong, Peters, Kafer, Braun 2001 Biol Reprod 64:1784−1789). Our objective is to identify the elusive factor that binds the TCE, a critical first step in understanding the mechanism of Prm1 mRNA repression.
Methods: Protein binding to the Prm1 TCE was analyzed with modified UV−crosslinking assays, RNA−affinity chromatography and mass spectrometry sequencing.
Results: Y−box protein 2, YBX2/MSY2, binds a previously unrecognized cis−element, GCCACCU, in the Prm1 TCE and a similar element in the Tnp1 3'UTR.
Conclusions: Our findings suggest that YBX2, a well−documented translational repressor, inactivates translation by binding directly to Prm−Tnp mRNAs. These findings agree with biochemical and gene knockout studies demonstrating that YBX2 is the predominant Y−box protein isoform in spermatids and the critical Y−box protein isoform for spermatid differentiation. YBX2 appears to repress the Prm1 mRNA by a novel mechanism because repression requires a YBX2−binding site near the 3' end of the Prm1 3'UTR and repressed Prm1 mRNAs have long poly(A) tails. YBX2 likely regulates many mRNAs, because it is a very abundant protein that binds a 7 nt element with four degenerate sites, [ACGU][CA]CA[UC]C[ACU], which appears near the 3' ends of other repressed mRNAs. The functions of YBX2 in spermatid differentiation are of special interest because single nucleotide mutations in the humanYBX2 gene correlate with abnormal protamine expression and male infertility. Thus, the mouse Ybx2 gene is an attractive model for elucidating how mutations in a regulatory factor cause defects in sperm development.

ABSTRACTS

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KDM1A OVEREXPRESSION IN MOUSE TESTES ALTERS THE EPIGENETIC LANDSCAPE OF SPERM HISTONES AND IS IMPLICATED IN TRANSGENERATIONAL INHERITANCE

Keith Siklenka, Serap Erkek[1], Maren Godmann[2], Romain Lambrot[2], Christine Lafleur[2], George Chountalos[2], Tamara Cohen[2], Marilene Paquet[2], Matthew Suderman[2], Mike Hallett[2], Serge McGraw[2], Donovan Chan[2], Jacquetta Trasler[2], Antoine Peters[1] and Sarah Kimmins[2]
[1]Friedrich Miescher Institute for Biomedical Research, Switzerland; [2]McGill University, Canada
(Presented By: Keith Siklenka)

Introduction: Sperm histones, previously thought to be retained at random and without function, are associated with CpG islands and hypomethylated DNA (Erkek et al 2013). Moreover, activating histone modifications, such as histone 3 lysine 4 (H3−K4) methylation, were found localized to promoters of genes implicated in embryonic development (Brykczynska et al 2010; Hammoud et al 2009). We hypothesize that the epigenetic marks on retained sperm histones serve to influence the health and development of offspring.
Methods: Therefore, we altered the mouse sperm epigenome through overexpression of the histone demethylase KDM1A specifically in the testes.
Results: Characterization of offspring sired by KDM1A+/− males revealed reduced survivability and a range of developmental defects. Importantly, this phenotype was also observed in offspring sired by descendants of transgenic fathers that did not inherit a transgenic allele. The observable abnormalities were cleared only in offspring sired by non−transgenic males with a transgenic great grandfather. These data suggests that inherited germ−cell epimutations may resist reprogramming for multiple generations before being reset. Analysis of the sperm epigenome of transgenic fathers by chromatin immunoprecipitation combined with genome sequencing (ChIP−Seq) revealed specific reductions of H3K4me2 at transcriptional start sites of over 2000 genes. Gene ontology analysis of these regions showed significant enrichment for genes associated with metabolic process, development and patterning. Moreover, Sequenome MassArray was used to analyze DNA methylation of transgenic and non−transgenic sperm at candidate genes; however, no significant differences were observed.
Conclusion: Genome wide DNA methylation analysis is ongoing. We show that sperm histone modifications, particularly H3K4me2, are important for guiding offspring development across multiple generations. Examination of alternate histone modifications such as H3K9me and H3K27me may shed light on how an altered sperm epigenetic landscape contributes to epigenetic inheritance.

4

CHRONIC EXPOSURE TO LOW DOSES OF DI−N−BUTYL PHTHALATE (DBP) RESULTS IN SMALLER TESTES, ABNORMAL TESTOSTERONE LEVELS, IMPAIRED BONE HEALTH AND GREATER WEIGHT GAIN IN ADULT MICE.

Sarah Moody, BBiomedSci[1], Hoey Goh, BBiomedSci[1], Rachelle Johnson, PhD[2], Natalie Sims, PhD[2], Kate Loveland, PhD[1] and Catherine Itman, PhD[3]
[1]Monash University; [2]St. Vincent's Institute; [3]University of Newcastle
(Presented By: Catherine Itman, PhD)

Introduction and Objectives: Phthalate esters are endocrine disrupting chemicals, which are linked to abnormal testis development. Animal studies typically use high doses (100−500 mg/kg/day) to study the consequences of phthalate exposure, however, our recently published data using the mouse have identified negative impacts on prepubertal development and androgen production at doses as low as 1 mg/kg/day (Moody et al 2013). The objective of this study was to determine whether chronic, low dose phthalate exposure affects body and testis weight and serum testosterone levels in adult mice. Because testosterone has important endocrine functions, we assessed bone density to identify the impact of DBP on non−reproductive organs.
Methods: C57Bl/6J mice were fed 1−10 mg/kg/day di−n−butyl phthalate (DBP) in corn oil vehicle, or vehicle only, from 4 − 21 days post partum (dpp) (prepubertal exposure) or 4−60 dpp (life−long exposure) (n>5/group). Mice were killed at 60 dpp and body and organ weights were measured. Serum testosterone levels were assessed by radioimmunoassay (Immunotech) and bone parameters by dual−energy X−ray absorptiometry (PIXImus).
Results: Adult DBP−fed mice gained more weight (~11−fold increase relative to weight at start of treatment) than vehicle−treated animals (9−fold increase, $P<0.05$), however, final body weights were not different between groups. DBP−fed mice had smaller testis to body ratios compared to mice fed corn oil (3.0−3.2 mg/g versus 3.65 mg/g, $P<0.05$) and had highly variable serum testosterone concentrations, with levels in the 10 mg/kg/day treatment group significantly higher than those of untreated animals (4227+/−1166 pg/ml versus 465+/−162 pg/ml, $P<0.05$). Mice administered 1 or 10 mg/kg/day DBP had lower bone mineral content and bone mineral density compared to mice fed corn oil.
Conclusion: This is the first study to link chronic low−level phthalate exposure with smaller adult testis size, greater weight gain and poor bone health parameters in mice, demonstrating the potential for phthalates to impact upon reproductive capacity and general health and well-being. Elevated serum testosterone in mice fed 10 mg/kg/day DBP was unexpected and may reflect abnormalities in Leydig cell function or testosterone metabolism. These data are particularly pertinent to studies in humans that have linked elevated urinary phthalate metabolite concentrations to metabolic syndrome, osteoporosis and impaired male reproductive function.

ABSTRACTS

5

PRENATAL EXPOSURE TO AN ENVIRONMENTALLY−RELEVANT CONTAMINANT MIXTURE ALTERS THE EPIGENOME OF FATHERS, DECREASES THEIR FERTILITY AND THE HEALTH OF THEIR SONS IN A RAT MODEL.

Clotilde Maurice, PhD Student[1], Serge McGraw, PhD[2], Arnaud Droit, PhD[1], Jacquetta Trasler, MD, PhD[2], Sarah Kimmins, PhD[2] and Janice Bailey, PhD[1]
[1]Université Laval; [2]McGill University
(Presented By: Clotilde Maurice, PhD Student)

Introduction: The Arctic food web is contaminated with organochlorines (OC) and Inuit populations have high OC body burdens. The health status of northern Inuit is poor relative to other Canadians and OC exposure might contribute to this discrepancy. We hypothesized that prenatal exposure to an environmentally−relevant OC mixture affects the paternal epigenome and his offspring's health.
Methods: Sprague−Dawley female rats (F0) were gavaged for five weeks with an environmentally−relevant concentration of an OC mixture or corn oil (Control) and mated to untreated males. Gavage continued until parturition of F1 litters. After weaning, F1 male pups were fed commercial chow and never directly exposed to OC. Adult F1 males (n=15) were mated to untreated females to generate F2 fetuses and pups; F2 development was followed until 90 days of age. To determine if prenatal OC exposure alters the paternal epigenome, F1 sperm were analyzed by reduced representation bisulfite sequencing (RRBS) to obtain genome−wide information on DNA methylation. RRBS libraries (n=6) were used in paired−end sequencing in 1 lane of a HiSeq 2000 sequencer (Illumina). Analysis and statistics for differentially methylated regions (±≥20%) were conducted using Methylkit software.
Results: F1 OC males were subfertile (83 vs. 97% conception; $P<0.05$). Litters sired by F1 OC had more preimplantation loss compared to F1 Controls (4 vs. 1/litter; $P<0.05$). 20% F2 fetuses from the F1 OC fathers had situs inversus (vs. 4% for F2 Controls; $P<0.05$). Compared to F2 Controls, F2 OC offspring from prenatally−exposed F1 OC fathers had a slower growth rate, presumably due to their smaller placentae ($P<0.05$). Preliminary data indicate that prenatal OC exposure alters sperm DNA methylation. Hypermethylation was a key epigenetic change in regions involved in embryo development (F1 OC vs. Control; $P\leq0.05$) and might partly explain the developmental phenotype of F2 sons sired by prenatally−treated F1 OC fathers, although these data have yet to be validated.
Conclusion: In conclusion, our preliminary data indicate that prenatal paternal exposure to an environmentally−relevant OC mixture induces reproductive dysfunction as well as developmental pathologies in his offspring, possibly due to epimutations of the sperm DNA. Specifically, hypermethylation of F1 OC sperm genes seem to correspond to the developmental pathologies observed in the F2 OC offspring relative to Controls, and we are currently validating these results.
Financed by FQRNT & RQR.

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PRENATAL EXPOSURE TO A COMBINATION OF ENDOCRINE DISRUPTORS EXACERBATES EARLY AND LONG TERM EFFECTS ON MALE REPRODUCTIVE HEALTH AND DEVELOPMENT

Steven Jones, MSc[1], Annie Boisvert, MSc[2], Peter Thrane, BSc[3], Sade Francois, BSc[4] and Martine Culty, PhD[5]
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(Presented By: Steven Jones, MSc)

Introduction: The increased incidence of male reproductive abnormalities is believed to result from endocrine disruptor (ED) induced perturbations of developmental processes in fetal testes. From conception through adulthood, humans are exposed to a multitude of anthropogenic and naturally occurring EDs. Few studies however, have evaluated the real life risk of exposure to ED mixtures at environmentally relevant doses on male reproductive health. We hypothesize that early life exposure to a low dose combination of Genistein (GEN), a soy derived phytoestrogen, and DEHP, an anti−androgenic plasticizer, will induce alterations in testes in a manner that is different from individual compounds.
Methods: Pregnant Sprague Dawley dams were gavaged from gestational day 14 to birth with either corn oil, genistein, DEHP or their mixture at 10 mg/kg/day. Testis development and function was subsequently examined in neonatal and adult male offspring. Testis weight of PND120 rats was significantly increased only in rats exposed to the mixture, while testosterone, LH and FSH were unchanged. Quantitative real time PCR analysis of PND120 testes showed increased expression of mast cell markers, suggesting inflammatory events in the testes of adult rats exposed to the mixture. The expression of the Sertoli cell marker WT−1 and germ cell−specific genes, including C−kit and Sox−17 showed significant decreases unique to combined exposure. Gene expression arrays of PND120 testes also revealed underlying genetic changes that were further validated by qPCR and IHC, confirming that fetal exposure to the mixture generated long term alterations in Leydig and germ cells.
Results: Analysis of in utero exposed PND3 and 6 testes also revealed significant aberrations in the mRNA expression of germ and somatic cell markers, and decreased protein expression of FOXO1 and PLZF in gonocytes. Similar changes were observed in an ex vivo culture system in which testis fragments were treated with MEHP, the principal bioactive metabolite of DEHP, alone or mixed with GEN.
Conclusion: These results demonstrate the ability of environmentally relevant mixtures of EDs to induce short and long term alterations in testicular gene expression and histology that are substantially different from those observed with individual exposures. Thus, assessing reproductive risk based on single chemical effects might not faithfully represent the true risks of exposure to low levels of ED mixtures during critical periods of male reproductive development.

ABSTRACTS

Sunday, April 6, 2014
2:00 p.m. - 3:30 p.m.

Concurrent Oral Session II: Human Spermatogenesis: Novel Findings in 2014
Location: Hope
Session Chairs:
Dolores J. Lamb, PhD, and Kyle Orwig, PhD

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LEVELS OF THE RETINOIC ACID SYNTHESIZING ENZYME ALDH1A2 ARE LOWER IN TESTICULAR TISSUE FROM MEN WITH INFERTILITY

John Amory, MD, MPH[1], Samuel Arnold, MS[1], Maria Lardone, MS[2], Antonio Piottante, MD[3], Mauricio Ebensperger, MD[4], Nina Isoherranen, PhD[1], Charles Muller, PhD[1], Thomas Walsh, MD[1] and Andrea Castro, MS[2]
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(Presented By: John Amory, MD, MPH)

Objective: As retinoic acid is necessary for spermatogenesis, we sought to determine if testicular levels of enzymes involved in retinoic acid biosynthesis were associated with male infertility.
Methods: Retrospective cohort. Testicular tissue samples from 32 infertile men and 11 controls seen at several infertility centers in Chile. Measurement of the three enzymes involved in retinoic acid biosynthesis, ALDH1a1, 1a2 and 1a3 in testicular tissue by a novel LC/MS/MS peptide assay. Enzyme levels were compared by type of infertility and correlated with testicular germ cell numbers, sperm parameters, serum and intratesticular hormone concentrations.
Results: Men with infertility had significantly reduced levels of ALDH1a2, but not ALDH1a1 or 1a3 in their testicular tissue compared to men with normal spermatogenesis. ALDH1a2 protein level was strongly correlated with the number of germ cells on testicular biopsy.
Conclusions: These findings suggest that ALDH1a2 is the main enzyme involved in retinoic acid biosynthesis in human germ cells. Further study of the relationship between intratesticular ALDH1a2 and male infertility is warranted to determine if men with infertility have a reduced ability to synthesize retinoic acid within their germ cells.

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A MICROARRY ANALYSIS OF UNIQUE GENES FOUND IN MEN WITH NON−OBSTRUCTIVE AZOOSPERMIA (NOA) AND VARICOCELES.

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Baylor College of Medicine
(Presented By: Jason Kovac, MD, PhD)

Introduction: Varicocele repair in men with NOA, or lack of sperm in the ejaculate, can result in improved spermatogenesis and ultimately, pregnancies achieved either naturally or via artificial reproductive technologies. The genetic difference between NOA men with and without varicoceles has never been reported. Results may yield importance information about the nature of the testicular changes seen in NOA men.
Methods: Tissues and blood were obtained from men with NOA (n=16) and subdivided into those with varicoceles (n=9) and those without (n=7). Gene−expression microarray (Agilent Sureprint G3) screened for genetic variations. Microarray data were evaluated with heatmaps, clustering and statistical analysis. Ingenuity Pathway Analysis (IPA) software using False Discovery Rates at 5% highlighted the candidate genes and pathways involved.
Results: Demographics showed control men and those with varicoceles to have similar ages (34±0.4 vs. 32±2 years) and testicular volumes (Left, 15±2 vs. 13±1 mL; Right, 14±2 vs. 13±1 mL). Serum levels for FSH (20±7 vs. 22±4 mIU/L), LH (7±1 vs. 8±1 mIU/L) and testosterone (313±43 vs. 296±30 ng/dL) were also identical between the groups. IPA revealed 44 genes preferentially expressed in men with varicoceles while network plotting identified 'Cellular Growth and Proliferation' as the most perturbed bio−function in NOA men with varicoceles ($p<0.05$, activation z−score 3.569). Genes most uniquely expressed in men with NOA and varicoceles included ANGPTL4 (induced under hypoxic conditions) and the CASP4 member of the caspase family (involved in apoptosis). IPA data−filtration also revealed that the serum biomarkers CAV1, CTSK, MCM7, NME1 and PLAT could be important in differentiating these patient populations.
Conclusions: The current study has identified several genes associated with the presence of varicoceles in men with NOA. Future studies will determine the use of both uniquely expressed genes and biomarkers to identify NOA patients more likely to benefit from varicocele repair.

ABSTRACTS

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MICRORNA EXPRESSION IN MEN WITH CONFIRMED DIAGNOSIS OF EARLY MATURATION ARREST

Ali Dabaja, MD, Anna Mielnik, MS, Matthew S. Wosnitzer, MD, Peter N. Schlegel, MD and Darius A. Paduch, MD, PhD
Weill Cornell Medical College
(Presented By: Ali Dabaja, MD)

Introduction: MicroRNAs (miRNAs) are short non−coding RNA molecules play a regulatory roles in expression of RNA transcripts. Recent studies indicate that miRNAs are mechanistically involved in the development of human spermatogenesis. However, little work has been done to compare the miRNA expression in men with normal fertility and in men with early maturation arrest (eMA). Our objective is to examine human miRNA expression in correlation with early eMA.
Methods: Testicular tissue from men with confirmed eMA diagnosis and normal spermatogenesis were analyzed. MicroRNA was isolated using the miRCURY™ RNA Purification Kit's based on spin column chromatography using a proprietary resin as the separation matrix. A miRCURY LNA™ Universal RT microRNA PCR system was used for sensitive and accurate detection of microRNA by quantitative real−time PCR. Statistical analysis was performed by GenEx V5.0.
Results: MicroRNA expression was determine for 13 normal fertile men and 4 men with the confirmed diagnosis of eMA, using the 96 well plates of previously selected primers that are relevant to testicular tissue. MiR−202−5p expression was reduced by 14 fold (P= 0.0001) in men with eMA comparing to normal. MiR−34c−5p was reduced by 40 fold (P=0.0023), miR−10b was reduced by 13 fold (p=0.0004), and miR−126−5p was reduced by 25 fold (p= 0.0024) in eMA comparing to normal fertile men. These differentially expressed microRNA have multiple target gene that effect quality control and turnover of cellular RNA, RNA transport, mTOR signaling pathway, Insulin signaling pathway, HIF−1 signaling pathway, and regulation of the spermatogenesis associated 22 gene.
Conclusion: Our results reveal an extended number of miRNAs that were differentially expressed in eMA males compared with normal fertile men. This data provide evidence for analysis of miRNA profiles as a future diagnosing, as well as a treatment tool for male infertility.

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MALE INFERTILITY FROM OVERUSE OF MEDICAL TESTOSTERONE IN MEN IN THIER REPRODUCTIVE YEARS – AN UNNECESSARY PROBLEM

William Parker, MD, Brian McArdle, DO, Arash Sattarin, Zachary Hamilton, MD and Ajay Nangia, MD
The University of Kansas
(Presented By: William Parker, MD)

Objective: To review the iatrogenic infertility caused by the use of medical testosterone in men of reproductive potential.
Methods: Men presenting with male infertility or hypogonadism in the reproductive years from 2008−2011 were studied. Analysis was performed of records of the patients on medical testosterone with respect to our treatment modalities and outcomes with respect to fertility and sperm recovery.
Results: During the study period, 548 patients met inclusion criteria for evaluation. Primary infertility was the predominant presenting complaint (69%) with oligospermia (35.7%) and azoospermia (26.5%) representing the majority of the semen analysis abnormalities (based on WHO 2010 criteria). Use of medical testosterone was present in 49 patients (8.9%). Of the 49 patients on testosterone, 24 presented for follow−up evaluation. 12 (50%) patients developed reproductive potential; 4 with a document pregnancy and 8 with sperm recovery. Average age in men who recovered was 37.6 (compared to 34.3; p=0.16). Among those with recovery, all had received prior intramuscular testosterone with a mean length of use of 27.5 months (compared to 67.1 months; p=0.19). Treatment choice consisted of human chorionic gonadotropin in 4, clomiphene citrate in 5, and discontinuation of testosterone in 3, with an average time to recovery of 8(3−24) months. In the 12 patients who failed to recover fertility at 14(5−25) months follow−up: 2 remained infertile despite therapy; 6 were lost to follow−up; 3 stopped treatment due to cost; and 1 reverted to testosterone.
Conclusion: Testosterone use in men of reproductive potential is a significant source of male factor infertility and can have devastating outcomes on future fertility. Not all men recover spermatogenesis despite literature supporting the reversibility of testosterone−induced spermatogenic suppression when used for contraception. Our experience highlights a vulnerable population of men and a need for improved education in the treatment of hypogonadism in the reproductive age.

ABSTRACTS

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POST−FINASTERIDE PERSISTENT SIDE EFFECTS MAY BE ASSOCIATED WITH PERSISTENT 5 ALPHA−REDUCTASE INHIBITION: A PILOT STUDY

Seth Cohen, MD, MPH
(Presented By: Seth Cohen, MD, MPH)

Introduction: Finasteride is an irreversible 5 alpha−reductase inhibitor (5ARi) used to treat both benign prostatic hyperplasia and androgenic alopecia. In some, finasteride use can be associated with sexual, cognitive and mood changes. The etiology of these side effects is unclear. During finasteride inhibition of 5AR, a lethal catalytic event, suicide substrate, occurs with a Ki = 1x1013M. Half−life for 5AR enzyme activity is reported to be 30 days, whereupon dihydrotestosterone (DHT) levels return to pre−5 ARi values. It is hypothesized that persistent side effects, months to years post−finasteride administration are associated, in part, with persistent 5AR inhibition, as measured by post−treatment DHT.

Methods: An IRB approved retrospective chart review was performed in 32 men examined between 2007−2013 who presented with side effects after finasteride use. Data collected included DHT, testosterone, sex hormone binding globulin, and calculated free testosterone values. Age of first use of finasteride, duration of use, and duration of persistent side effects were recorded. Data from psychometrically validated questionnaires were assessed for parametric data analysis. All data were analyzed using a Spearman's rank−order correlation.

Results: Mean duration of finasteride use was 41.8 +/− 49.9 months (range: 1 month to 13 years) and of persistent side effects was 37.0 +/− 33.4 months (range: 1 month to 10 years). Mean baseline values were: dihydrotestosterone: 29.8 ng/dl +/− 9.1 ng/dl, testosterone: 443.9 ng/dl +/− 109.8 ng/dl, and calculated free testosterone: 9.4 ng/dl +/− 3.6. Mean baseline erectile function score was 15.43 +/− 8.7 (min/max: 1−29); mean sexual desire score was 4.9 +/− 2.9 (min/max: 2−10), mean overall sexual satisfaction score was 4.0 +/− 2.5 (min/mx: 1−10). Using bivariate analysis, length of finasteride use correlated with decreased DHT values ($p < 0.046$) and duration of sexual side effects ($p<0.009$).

Conclusion: Side effects with finasteride use may persist even after discontinuation. Mechanistic hypotheses include persistent endocrine and epigenetic gene expression alterations of the 5AR enzyme induced by finasteride exposure. Patients should be informed of possible persistent side effects prior to starting finasteride. More research is needed.

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RECOVERY OF UNDIFFERENTIATED SPERMATOGONIA FROM THE TESTES OF PREPUBERTAL PATIENTS AFTER EXPOSURE TO CHEMOTHERAPY

Hanna Valli[1], Karen A. Peters[2], Brian P. Hermann[3], Meena Sukhwani[2], Peter H. Shaw[4], Joseph S. Sanfilippo[5], Thomas M. Jaffe[6] and Kyle E. Orwig[7]
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(Presented By: Hanna Valli)

Introduction: Spermatogonial stem cells (SSCs) have the potential to regenerate spermatogenesis in some cases of male infertility. Grown men and pubertal boys have the option to freeze sperm, but currently there are no options to preserve fertility for prepubertal patients rendered infertile by some types of cancer treatments. The spermatogonial stem cell transplantation approach has been shown to be successful in several animal models (mice, rats, pigs, goats, bulls, sheep, dogs and monkeys) and academic centers around the world are already cryopreserving testicular tissue for prepubertal patients, in anticipation that by the time these patients are ready to have children, the technology will be translated to the clinics.

Methods: Here we report on the harvesting of testicular tissue by biopsy from 6 patients (ages 2−12) who were considered at high risk for loss of future fertility due to treatment. Five of these patients had initiated chemotherapy prior to cryopreserving tissue.

Results: The amount of tissue obtained from testicular biopsy was between 77 and 962.8 mg per patient and enzymatic digestion with clinical grade enzymes yielded from 33.6 x 106 to 148.6 x 106 cells per gram of tissue. The presence of undifferentiated spermatogonia in all patient samples, including the five who had already initiated chemotherapy, was confirmed by staining with established human spermatogonia markers, SALL4, UTF1 and VASA. Quantification of UTF1 positive cells revealed that the patients had between 0.1 and 8.3 UTF1 positive cells per tubule.

Conclusion: This is the first report demonstrating that undifferentiated spermatogonia can be recovered from testicular tissue biopsies obtained from patients after starting a chemotherapy regimen.

This work was supported by NIH grants HD055475, HD061289 and HD008610, The Scaife Foundation, The Richard King Mellon Foundation, The US−Israel Binational Science Foundation and Magee−Womens Research Institute and Foundation.

ABSTRACTS

Sunday, April 6, 2014
11:00 a.m. - 12:30 p.m.

Poster Session I*
**Not CME Accredited*
Location: Venetian

1

LONG−TERM TREATMENT WITH TESTOSTERONE UNDECANOATE (TU) IN HYPOGONADAL MEN WITH CARDIOVASCULAR DISEASES (CVD): OBSERVATIONAL DATA FROM A REGISTRY STUDY

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[1]Bayer Pharma AG, Global Medical Affairs Andrology; [2]Private Urology Practice; [3]Boston University School of Public Health; [4]Boston University School of Medicine
(Presented By: Farid Saad, DVM, PhD)

Introduction: Hypogonadism is associated with cardiometabolic risk. Several studies suggest that hypogonadism increases the risk of all−cause and cardiovascular mortality. While some short−term studies have been performed in men with CVD, there are no data on long−term effects of testosterone replacement therapy (TRT) in men with CVD.
Methods: In a prospective, cumulative, observational registry study from a single urologist's office, 300 men with testosterone ≤12.1 nmol/L received TU injections for up to 6 years. In this subgroup analysis, 68 men with a previous diagnoses of coronary artery disease (CAD; n=40) and/or a history of myocardial infarction (MI; n=40) were analyzed.
Results: Mean age was 60.76±4.94 years. 68 men were included for 2 years, 59 for 3 years, 54 for 4 years, 44 for 5 years and 28 for 6 years. Declining numbers reflect the nature of the registry (patients are included after receiving 1 year of TRT) but not drop−out rates. Weight (kg) decreased from 115.07±13.71 to 92.5±9.64. Waist circumference (cm) decreased from 112.07±7.97 to 99.89±6.86. BMI decreased from 37.27±4.45 to 30.14±3.21 ($p<0.0001$ for all). Mean weight loss was 17.05±0.57%. Mean fasting glucose decreased from 108.74±17.08 to 96.0±1.92 mg/dl, HbA1c from 7.81±1.17 to 6.2±0.62% ($p<0.0001$ for both). Total cholesterol decreased from 304.66±34.09 to 189.32±9.68, LDL from 184.28±37.51 to 134±27.91, triglycerides from 308.38±56.3 to 187.71±8.67 ($p<0.0001$ for all) and HDL increased slightly. The total cholesterol:HDL ratio declined from 5.16±1.55 to 3.15±0.87 ($p<0.0001$). Systolic BP decreased from 167.82±11.01 to 142.36±10.62, diastolic BP from 102.28±8.23 to 81.25±8.07 mmHg ($p<0.0001$ for both). Pulse pressure declined from 65.54±5.24 to 61.11±4.66 ($p<0.0001$). Quality of life, measured by the Aging Males' Symptoms scale (AMS) improved from 56.25±10.09 to 17.11±0.31. The minimum number of injections was 9, maximum 26. In no patient TRT was discontinued or interrupted. There were no major cardiovascular events during the observation time.

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156 HYPOGONADAL MEN WITH OBESITY AND TYPE 2 DIABETES ACHIEVE WEIGHT LOSS AND IMPROVED GLYCAEMIC CONTROL UPON TREATMENT WITH TESTOSTERONE UNDECANOATE UP TO 6 YEARS: A SUBGROUP ANALYSIS FROM TWO OBSERVATIONAL REGISTRY STUDIES

Farid Saad, DVM, PhD[1], Ahmad Haider, MD, PhD[2], Aksam Yassin, MD, PhD[3], Gheorghe Doros, PhD[4] and Abdulmaged Traish, PhD[5]
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(Presented By: Farid Saad, DVM, PhD)

Introduction: Obesity is a major risk factor for type 2 diabetes (T2D). In men, both diseases have a high prevalence of testosterone deficiency (hypogonadism). Testosterone replacement treatment (TRT) has been shown to improve weight and T2D. Numerous mechanisms have been identified as to how testosterone impacts glycaemic control. We studied the effects of TRT in obese hypogonadal men with T2D.
Methods: Cumulative, prospective, observational registry studies of 561 hypogonadal men from two urological centers. From these registries, we selected all men with obesity and T2D for subgroup analysis. All men received testosterone undecanoate injections for up to six years. All men were treated for their T2D by their respective family physician.
Results: 156 men (28% of all patients) met our criteria. Mean age was 61.2±6.2 years at start of treatment. Weight (kg) decreased from 113.56±11.53 to 97.18±9.04. This decrease was statistically significant vs baseline ($p<0.0001$) and each year compared to previous year. The model−adjusted mean change from baseline was −17.49±0.58 kg. The mean per cent weight loss (%) was 15.04±0.48 after 6 years. Waist circumference (cm) declined from 114±8.69 to 102.52±7.93. This was statistically significant vs baseline ($p<0.0001$) and each year compared to the previous. The mean change from baseline was −11.56±0.34 cm. BMI (kg/m2) decreased from 36.31±3.51 to 31.19±2.6. This change was statistically significant vs baseline ($p<0.0001$) and each year compared to previous year. The mean change from baseline was −5.59±0.18 kg/m2. Fasting glucose (mg/dl) decreased from 128.37±31.63 to 101.55±17.02 ($p<0.0001$ vs. baseline, significant for the first two years vs. previous year). The mean change from baseline was −27.14±2.48 mg/dl. HbA1c decreased from 8.08±0.9 to 6.14±0.71% ($p<0.0001$ vs. baseline, significant for the first 5 years vs. previous year and approaching significance from year 6 to year 5 at $p=0.0635$). The mean change from baseline was −1.93±0.06%. At baseline, 25 (16%) of all patients had an HbA1c ≤ 7.0% and 12 (7.7%) an HbA1c ≤ 6.5%. At the end of the observation period, 128 (82.05%) had reached an HbA1c target of ≤ 7.0% and 106 (67.95%) an HbA1c target of ≤ 6.5%.
Conclusions: Correcting hypogonadism by TRT with testosterone undecanoate injections in obese hypogonadal men with T2D resulted in significant and sustained improvements in weight, waist circumference, fasting glucose and HbA1c over the full 6 years of the study.

ABSTRACTS

3

LACK OF ACTIVATION OF ENCLOMID TO ITS 4−HYDROXYLATED FORM BY CYP 2D6 DOES NOT EXPLAIN LACK OF TESTOSTERONE RESPONSE

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Repros Therapeutics Inc.; The Woodlands TX 77381
(Presented By: Ronald Wiehle, PhD)

Introduction and Objective: Enclomid (Androxal), an isomer of Clomid, is effective in raising serum testosterone (T) in ~80% of men with secondary hypogonadism. The drug appears to act at the level of the hypothalamus/pituitary by first raising LH and FSH. We hypothesized that those few men who do not respond with an increase in serum T could have a defective CYP 2D6 which does not allow the metabolism of the parent drug to the highly active 4−hydroxy−Enclomid form. To determine the proportion of men with secondary hypogonadism that are non−responders to Enclomid who also do not make 4−hydroxy−Enclomid.

Methods: A Phase 3 clinical trial (ZA−302) in men with secondary hypogonadism. Subjects were enrolled through the criteria of two morning serum T in the hypogonadal range and normal LH levels. All subjects were treated with 12.5 or 25 mg of Enclomid daily and orally for 12 weeks. Men were assessed for serum LH, FSH, and serum T. The trough (steady state) levels of serum Enclomid and 4−OH−Enclomid were determined by HPLC at the end of the study.

Results: Eight−one percent of men attained morning serum T in the 300−1000 ng/dL range. We looked a subset of 12 men who were non−responders in terms of serum T and 22 other men who did respond. Most men demonstrated high conversion of Enclomid to 4−hydroxy−Enclomid such that the ratio of 4−Hydroxy−Enclomid to Enclomid was 1.4 (+/− 0.78). Looking at 12 non−responders, we determined that only 2 individuals showed low levels of 4−Hydroxy−Enclomid but one man out of 22 who did respond with a higher T level also showed low 4−hydroxy−Enclomid. Essentially all men demonstrated increase in serum LH.

Conclusions: We infer that 4−hydroxy−Enclomid is probably not required for hypothalamic−pituitary release of LH and the metabolite is not necessary for increasing serum T. The inability to raise T despite increasing LH suggests an additional factor is involved. This results needs to be verified in a larger data set and attention to other metabolites. This work was supported by Repros Therapeutics.

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ESTRADIOL INCREASES THE PROLIFERATION OF RAT IMMATURE LEYDIG CELLS: A POSSIBLE ROLE FOR LEYDIG CELL TUMOR FORMATION

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(Presented By: Xiaoheng Li)

Introduction: The causes of Leydig cell tumor formation are still unclear. Estradiol (E2) has been hypothesized to cause Leydig cell tumor. However, its mechanism is unknown. Unlike adult rat Leydig cells that have no proliferative capacity, rat progenitor and immature Leydig cells have the higher proliferative ability.

Methods: In the present study, rat progenitor, immature and adult Leydig cells were isolated from the testes of 21, 35 and 90 day old Sprague Dawley rats, and treated with different concentrations of E2. After 24 hours of treatment, these cells were incorporated with a radio−labelled thymidine. E2 did not affect the proliferative capacity of rat progenitor and adult Leydig cells, while it significantly increased the thymidine incorporation into immature Leydig cells.

Results: The incorporation rates to immature Leydig cells were significantly increased by52,120, 123, and 364% after 50, 250, 500 and 1000 nM E2 treatment, respectively. Immature Leydig cells had significantly higher estrogen receptor α expression when compared to progenitor and adult Leydig cells. Whole genomic profiling analysis showed that E2 significantly upregulated neuropeptide Y receptor and insulin−like growth factor 2 receptor signalling pathway.

Conclusion: In conclusion, at higher concentration, E2 can stimulate the proliferation of rat immature Leydig cells.

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PLATELET−DERIVED GROWTH FACTOR (PDGF) STIMULATES DIFFERENTIATION OF RAT IMMATURE LEYDIG CELLS VIA INCREASING THE EXPRESSION OF STAR

Xiaomin Chen, PhD[1], Xiaoheng Li, MS[2], Kaimin Yuan, PhD[3], Shiwen Liu, MS[4], Tiao Bu, MS[4], Qiufan Wang, PhD[5], Qingquan Lian, PhD[5], Ren−Shan Ge, PhD[5] and Guimin Wang, PhD[5]
[1]Research assistant; [2]Laboratory technician; [3]Attending doctor; [4]Master student; [5]Professor
(Presented By: Xiaomin Chen, PhD)

Introduction: Platelet−derived growth factor (PDGF) is one of growth factors that regulate cell growth and differentiation. In the lineage of rat Leydig cells, there is an increased expression of the α receptor (PDGFRA) during pubertal development. However, the mechanism of PDGF in the regulation of Leydig cell development is unclear.

Methods: In the present study, rat immature Leydig cells were isolated from the testes of 35−day−old Sprague Dawley rats, and treated with 1 and 10 ng/ml of PDGF−BB.

Results: After 24 hours of treatment, these cells were harvested for genomics profiling and the medium steroids were measured. 1 and 10 ng/ml PDGF−BB significantly increased androgen production by rat immature Leydig cells.

Conclusion: Genomics profiling analysis showed that the expression levels of steroidogenic acute regulatory protein (Star) were increased by 2−fold. Further analysis showed that Egr1 and Egr2 expression levels were increased 4.9 and 3.6 fold by 10 ng/ml PDGF−BB, respectively. In conclusion, PDGF−BB stimulated the differentiation of rat immature Leydig cells via regulating Star.

ABSTRACTS

6

TESTOSTERONE AS PROGNOSTIC INDEX IN ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Sebastiano Raimondo, Trainee[1], Alessandro Di Marco Berardino, Trainee[2], Chantal Di Segni, Trainee[1], Riccardo Inchingolo, MD[2], Andrea Smargiassi, MD[2], Salvatore Valente, MD[2], Giuseppe Maria Corbo, MD[2], Alfredo Pontecorvi, MD[1] and Antonio Mancini, MD[1]
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(Presented By: Antonio Mancini, MD)

Introduction: Today chronic obstructive pulmonary disease (COPD) is not considered only a lung disease, in fact, systemic comorbidities, like weight loss, have not secondary role in evolution of the disease. Exacerbation of COPD (AECOPD) negatively influenced the natural history of the illness and it has been found related to muscle dysfunction. In this pathway, hypogonadism could play a pivotal role.
Methods: Our study want to evaluate possible relationships among prognostic indexes of AECOPD (APACHE II Score), inflammation (serum amyloid A, SSA) and hormonal axes primarily involved in metabolic balance of COPD patients. 24 patients, aged 75 ± 13 yrs, 17 males, were studied. Descriptive statistical analysis shows reduced values of testosterone (T) (1.85 ± 2.28 ng/mL), free testostosterone (f−T) (0.028 ± 0.030 ng/mL), dihydrotestostrone (DHT) (0.18 ± 0.19 ng/mL) and IGF−1 (91.840 ± 74.19 pg/mL).
Results: Frequency distributions of Apache II score and SSA were calculated and, using tertile as cut off point, three categories were made and used in the analysis (SSA: = 8 mg/mL; 9−160 mg/mL; = 160 mg/mL); (APACHE II: = 10; 11−12; = 12). Using this classification, an inverse correlation between SAA and T (p: 0.01), f−T (0.01), DHT (0.001) and IGF−1 (p: 0.05) was found.
Conclusion: Data show the same inverse relationship between APACHE II tertiles on one hand and T (p: 0.01) and f−T (p: 0.02) on the other hand. Even if we cannot establish a causal relationship between hypogonadism and severity of disease, our data suggest systemic effects of AECOPD and a possible mechanism explaining wasting syndrome.

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INFLUENCE OF TESTOSTERONE DEPRIVATION ON OXIDATIVE STRESS INDUCED NEURONAL DAMAGE IN HIPPOCAMPUS OF ADULT RATS

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(Presented By: Prakash Seppan, PhD)

Introduction and Objective: Increasing evidence supports the role for androgens in brain function, through genomic and non−genomic mechanisms. Analyzes the testosterone action in hippocampus can lead towards identifying therapeutic targets for not only reproductive function, but also adult sexual behavior and cognition. Due to the functional and high energy demand in hippocampal neurons, increased reactive oxygen species is a common factor and hence requires very good anti−oxidant system, together the role of testosterone in these neuronal cells seem to be imperative in this process. To study the influence of testosterone deprivation induced oxidative stress and the cascade hippocampal cell damage, it's possible impact on memory and cognitive behavior.
Methods: Adult male Wistar albino rats were used as control, castrated and castrated + testosterone supplemented (5mg/Kg/day) groups. From 10th day after surgery, the animals were subjected to analysis of pituitary−testicular axis by estimating serum testosterone, FSH and LH. Assessment for memory using radial arm maze and affective behavior assessment was done by open−field test and elevated plus maze. By 18th day animals were sacrificed. Hippocampus processed for biochemical analyses of SOD, GPX, GR, Catalase, LPO, GST, Vit C and Vit E. Histology analyzed using H&E staining.
Results: Following castration, pituitary testicular axis was disrupted. Significant reduction of both enzymic and non−enzymic antioxidant levels were observed in castrated animal hippocampus. Memory acquisition was also significantly reduced in castrated animals. Anxiety and affective behavioral changes were more pronounced in castrated animals compared to the intact animals. Histological sections of hippocampus showed degenerative changes in the nuclear layer of CA3 and CA4 area of hippocampus. Above alteration were reverted to normal state as that of control animals in castrated +testosterone group. **Conclusion:** It was evident it is the testosterone deficiency that induces oxidative stress in the hippocampus and clearly affecting its physiological functions as well as its anatomical integrity. Physiological testosterone therapy is able to suppress oxidative stress probably mediated via the AR−independent or dependent pathway, indicating critical role of testosterone in neuro−biology. This requires further study to understand their complex relationship(s).

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EFFECTS OF FOUR CHEMOTHERAPEUTIC AGENTS, BLEOMYCIN, ETOPOSIDE, CISPLATIN AND CYCLOPHOSPHAMIDE, ON DNA DAMAGE AND TELOMERES IN A MOUSE SPERMATOGONIAL CELL LINE

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(Presented By: Mingxi Liu, PhD)

Introduction: Treatment with chemotherapeutics agents may induce persistent DNA damage in male germ cells with the possibility of long term consequences on fertility and progeny outcome. Telomeres, specialized structures at the physical ends of chromosomes, play an important role in the maintenance of genetic stability and in the response of somatic cells to anticancer drugs.

Methods: Our objective was to test the hypothesis that exposure to bleomycin, etoposide, or cisplatin (the drug regimen used to treat testicular cancer) or cyclophosphamide (a commonly used anticancer agent and immunosuppressant) targets telomeres in the male germ line. C18−4 spermatogonial cells (a gift from Dr. MC Hofmann) were exposed to bleomycin, etoposide, cisplatin or 4−hydroperoxycyclophosphamide (4−OOHCPA, a pre−activated analog) in vitro. DNA damage was assessed by γH2AX immunofluorescence. Telomeres were detected by fluorescence in situ hybridization (FISH) using a telomeric Cy3−conjugated peptide nucleic acid (PNA) probe. The extent to which DNA damage was localized in telomeres was analyzed with Imaris software. Telomere length (the ratio of telomere repeat copy number to single copy gene copy number) was assessed using q−PCR, telomerase activity was determined with the telomere repeat amplification protocol (TRAP) assay, and steady state concentrations of the mRNAs for telomerase enzyme components, Tert and Terc, by qRT−PCR analysis.
Results: All four anticancer drugs induced a significant increase in γH2AX immunofluorescence in C18−4 cells. Interestingly, the γH2AX signal was localized to telomeres after treatment with bleomycin, cisplatin, and 4−OOHCPA, but not etoposide. Mean telomere lengths, the intensity of the telomere FISH signal, telomerase activity, and the expression of Tert and Terc were reduced by exposure to cisplatin and 4−OOHCPA, but not by bleomycin or etoposide.
Conclusion: Thus, although all four anticancer drugs induce DNA damage in this spermatogonial cell line, only cisplatin and 4−OOHCPA, the two alkylating agents, induce telomere dysfunction. This telomere dysfunction may contribute to infertility and developmental defects in the offspring.
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EFFECT OF ROSMARINIC ACID ON SERTOLI CELLS APOPTOSIS AND SERUM ANTIOXIDANT LEVELS IN RATS AFTER EXPOSURE TO ELECTROMAGNETIC FIELDS

Arash Khaki, DVM, PhD
(Presented By: Arash Khaki, DVM, PhD)

Introduction and Objective: Rosmarinic acid belongs to the group of polyphenols; it has antioxidant, anti−inflammatory and antimicrobial activities and help to prevent cell damage caused by free radicals. The objective was to study the effect of Rosmarinic acid on sertolli cells apoptosis and serum antioxidant levels in rats after they were exposed to electromagnetic fields.
Methods: Male Wistar rats (n=40) were allocated into three groups: control group (n=10) that received 5cc normal saline (0.9% NaCl) daily by gavage method, Rosmarinic acid group that received 5mg/rat (gavage) (n=10), electromagnetic fields (EMF) group that had exposure with 50hz (n=20) which was subdivided to two groups of 10; EMF group and treatment group. Treatment group received 5mg/rat (gavage) Rosmarinic acid daily for 6weeks, respectively. However, the control group just received an equal volume of distilled water daily (gavage).
Results: On the 42nd day of research, 5cc blood was collected to measure testosterone hormones, total antioxidant capacity (TAC), levels from whole group's analysis. Level of malondialdehyde (MDA) levels and sertoli cells apoptosis significantly decreased in the group that received 5mg/rat of Rosmarinic acid ($P<0.05$) in comparison with experimental groups. Level of testosterone, total antioxidant capacity (TAC), significantly increased in groups that received Rosmarinic acid ($P<0.05$).
Conclusion: Since in our study 5mg/rat of Rosmarinic acid showed significantly preventive effect on cell damages especial sertoli cells apoptosis that caused with EMF, it seems that using Rosmarinic acid as food additive can be effective for supporting people living under EMF environmental pollution.
Keywords: Apoptosis, EMF, Rosmarinic acid, Sertoli cells, Testosterone.

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HUMAN SPERM BIOASSAY IN EVALUATING THE QUALITY OF BLOOD SERUM AND FOLLICULAR FLUID OF FEMALES UNDERGOING IN VITRO FERTILIZATION (IVF) BASED INFERTILITY TREATMENT

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(Presented By: Amjad Hossain, PhD)

Objectives: Human sperm bioassay (HSB) is a convenient in−house quality control test in laboratories that are involved with assisted reproductive technology based fertility treatment. Proficiency test providers also take advantage of human sperm for developing proficiency test (PT). In ovarian stimulation with gonadotropins, gonadal secretions accumulate in follicular fluid (FF) and also in blood serum (BS). In this study, the ability of HSB in evaluating the variation in the quality of FF and BS of females undergoing IVF was assessed.
Methods: BS and FF were obtained from IVF patients. The samples were representative of 3 conditions: patient age (young vs old), ovarian response (normal, poor and high) and procedure outcome (pregnant vs non−pregnant). Embryo culture media (ECM) and supplement (serum albumin, SA) obtained from American Association of Bioanalysts (AAB) as PT sample was used to prepare control. ECM was also used as the base media in the experimental group. Conventional HSB was performed following the method provided by AAB. Briefly, 0.5 ml ECM was supplemented with SA, BS or FF at 5%. The culture medium was maintained in center well dish (Falcon) covered with 1 mL oil (Irvine). The sperm concentration was adjusted to 3x106/ ml in the culture and the culture dishes were kept in the incubator (37o C and 5.5% CO2). Sperm motility and motility grade were determined at 0 and 48 hour. In motility grade evaluation, only grades 3 and 4 were taken into consideration.
Results: By 48 hrs culture, the decline in motility grade was more stringent than that in motility (90% to 30% vs. 90% to 60%). Cultures supplemented with BS and FF exhibited a trend of higher motility compared to that of SA supplemented controls but no difference between them (SA 56+ 3%, BS 64+ 2 %, FF 64+ 3%). Similarly, as assessed by sperm motility, the cultures did not discriminate age (< 35 yrs 65+ 2 % vs > 35 yrs 64+ 2%), ovarian response (poor 64+ 3% vs normal 63+ 3% vs high 64+ 2%) and pregnancy potential (pregnant 64+ 3% vs non−pregnant 63+ 2%). Motility grade imitate the motility pattern in respective culture conditions.

Conclusion: There occurred increased deterioration in motility grade compared to motility. The sensitivity of the conventional human sperm bioassay was not strong enough in revealing the fine differences in the quality of the human body fluid such as BS and FF. The low sensitivity is probably attributable to the assay procedure.

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EFFECTS OF APIGENIN ON THE DEVELOPMENT AND FUNCTION OF RAT IMMATURE LEYDIG CELLS

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(Presented By: Qiqi Zhu, MA)

Introduction: Apigenin is a natural flavone. However, whether it interferes with the androgen production in Leydig cells is unclear. The object of the present study was to investigate the effects of apigenin on the development and function of rat immature Leydig cells.
Methods: Rat immature Leydig cells were incubated for 3 hours with 100 μM without (basal) or with 1 ng/ml luteinizing hormone (LH), 20 μM of the following chemicals: 8−bromoadenosine 3',5'−cyclic monophosphate (8BR), 22R−hydroxychloesterol (22R), pregnenolone (PREG), progesterone (P4), and androstenedione (D4). The medium level of 5α−Androstane−3α,17β−diol (DIOL), the primary androgen produced by rat immature Leydig cells, was measured.
Results: Apigenin significantly inhibited basal, 8BR, 22R, PREG, P4, and D4 stimulated DIOL production in rat immature Leydig cells. Further study showed that apigenin inhibited rat 3β−hydroxysteroid dehydrogenase, 17α−hydroxylase/17,20−lyase, and 17β−hydroxysteroid dehydrogenase 3 with IC50 values of 11.41± 0.7, 8.98± 0.10, and 9.37±0.07 μM, respectively. Apigenin inhibited human 3β−hydroxysteroid dehydrogenase and 17β−hydroxysteroid dehydrogenase 3 with IC50 values of 2.17 ± 0.04 and 1.31 ± 0.09 μM, respectively.
Conclusion: In conclusion, apigenin mainly inhibited rat and human steroidogenic enzymes.

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STIMULATION OF STEROIDOGENESIS IN RAT IMMATURE LEYDIG CELLS BY BROMINATED FLAME RETARDANT BDE−100

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(Presented By: Haiyun Deng, MD)

Introduction: Polybrominated diphenylether BDE−100 is considered as a potential endocrine disruptor. The objective of this study was to explore whether BDE−100 could affect androgen biosynthesis and metabolism in rat immature Leydig cells.
Methods: Rat immature Leydig cells (ILCs) were treated with 3×10−9 to 3×10−6 M BDE−100 in vitro for 3hr, the production of 5α−androstane−3α,17β−diol (DIOL), the primary androgen produced by rat immature Leydig cells and steroidogenic enzyme activities were determined.
Results: 3×10−6 M BED−100 significantly increased basal, LH−, 8bromo−cAMP−stimulated DIOL production by 2, 2, and 5 fold. At this concentration BED−100 did not affect 22R−OH−cholesterol and pregnenolone−stimulated DIOL production. Indeed, at this concentration BED−100 stimulated Scarb1 and Lhcgr expression levels of ILCs. However, it did not affect the expression levels of other Leydig cell genes, including Star, Tpso, Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b3, Srd5a1 and Ark14c.
Conclusion: The results of this study indicate that environment−related level of BDE−100 in vitro increased DIOL production in a dose−dependent manner. The stimulated effects of BDE−100 on Scarb1 and Lhcgr might play key roles in BDE−100−mediated stimulation of DIOL production.

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MONONUCLEAR PHAGOCYTES FROM THE PROXIMAL MOUSE EPIDIDYMIS TAKE UP LUMINAL BACTERIA.

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(Presented By: Tegan Smith, PhD)

Introduction: Our discovery of a dense and heterogeneous network of mononuclear phagocytes (MPs) in the murine epididymis raises questions regarding the function of these antigen−presenting cells, which express classic macrophage and dendritic cell markers including CD11c, F4/80 and CX3CR1. We hypothesize that one such function might be to contribute to the maintenance of a pathogen−free luminal environment, therefore the objective of this study was to assess the ability of epididymal MPs (eMPs) to take up Escherichia coli (E. coli), a bacterium known to induce inflammation in the human epididymis manifesting in epididymitis.
Methods: In this study, CX3CR1+ MPs isolated from the proximal mouse epididymis (initial segment and caput) were co−incubated with fluorescent E. coli bioparticles, fixed and assessed by fluorescence microscopy. After 2 hours co−incubation, CX3CR1+ cells were filled with E. coli particles, indicating that eMPs have very potent phagocytic capabilities in vitro. In order to determine the ability of eMPs to capture antigens in vivo, we have developed a micro−inoculation technique whereby soluble and insoluble materials can be administered into the lumen of the proximal epididymis via the efferent ducts, causing minimal damage and disruption to the epididymis.
Results: Four hours following injection of fluorescent E. coli, particles could be clearly visualized in the lumen of the proximal segments, and a number of E.coli particles were located within epithelial cells that did not express V−ATPase, indicating that principal cells phagocytosed E. coli particles. Very few particles were located within CD11c+ and CX3CR1+ MPs. However, 24 hours after micro−inoculation, E.coli particles were observed accumulating within CD11c+ and CX3CR1+ MPs located in the basal region of the epithelium.

Conclusion: Our results indicate that peritubular MPs from the proximal epididymis take up antigenic particles originated specifically from the luminal compartment. We are currently characterizing the uptake of other soluble and insoluble antigens, as well as the mechanisms that control antigen acquisition. The respective roles of epithelial cells and epithelium−associated mononuclear phagocytes in the epididymal mucosa remain to be elucidated.

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ROLE OF SPERM TRANSCRIPTS IN THE ETIOLOGY OF IDIOPATHIC RECURRENT EARLY PREGNANCY LOSS

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(Presented By: Kranthi Vemparala, PhD)

Introduction: Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy losses before the 20th week of gestation. Although multiple factors involved in the etiology of RSA, RSA is traditionally diagnosed from the maternal perspective and the role of paternal factors in recurrent abortion less understood. The relationship between sperm parameters and RSA is controversial and Molecular parameters like sperm DNA fragmentation index (DFI) are not sufficient in RSA diagnosis and still there is need to find other molecular factors which compliment DFI in better diagnosis. As paternal genome is has profound importance in fetal development, and our objective of this study is to understand the role of sperm gene expression in RSA.
Methods: Ejaculates were obtained from 24 fertile healthy volunteers and 24 male partners of couple experiencing idiopathic RSA. After routine Semen analysis, cDNA was synthesized using Total RNA extracted from separated Sperm cells and gene expression analyzed by qPCR. The genes TOMM7, RPS6, RBM9, RPL10A, EIF5A, AKAP4, FOXG1, Sox3, and STAT4 were selected for gene expression analysis based on previous literature and were validated in RSA patients.
Results: Out of 9 genes studied, Expression of 7 genes (TOMM7, RBM9, RPL10A, EIF5A, AKAP4, FOXG1, Sox3, and STAT4) was slightly upregulated, one gene (Sox3) was highly upregulated (3 fold) and for one gene no change in expression was observed compared to their counterparts. The Mean fold changes for TOMM7, RPS6, RBM9, RPL10A, EIF5A, FOXG1, Sox3, AKAP4, and STAT4 are 1.78, 1.05, 1.73, 1.91, 1.22, 2.16, 4.1, 1.66, and 2.34 respectively.
Conclusion: The genes which are regulators of protein synthesis, mitochondrial import, alternate splicing, Sperm fibrous sheath assembly, apoptosis and cell survival which are critical for normal embryo development are upregulated and Sox3 gene which is essential for normal Spermatogenesis is highly upregulated suggests the role of these genes in recurrent spontaneous abortion. But further functional studies would confirm and validate the usability of the expression profile of these genes in the molecular diagnosis of RSA to compliment already established indicators like DNA fragmentation index.

No	Gene Name	Mean fold change
1	TOMM7	1.775
2	RPS6	1.051
3	RBM9	1.727
4	RPL10A	1.912
5	EIF5A	1.221
6	FOXG1	2.156
7	SOX3	4.098
8	AKAP4	1.657
9	STAT4	2.338

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PRIMARY TESTICULAR FAILURE: GENOTYPE PHENOTYPE CORRELATION OF 140 CASES

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Introduction: Primary testicular failure (PTF) refers to conditions where testes fail to produce sperms despite adequate hormonal support. PTF is classified into four distinct subtypes viz., Sertoli Cell Only Syndrome (SCOS), Maturation Arrest (MA), Hypospermatogenesis (HS) and Tubular Fibrosis (TF). Despite efforts, causes of PTF in most cases are still unknown. This study is based on 140 apparent idiopathic PTF cases. Known causes viz., mumps orchitis, varicocele, torsion, trauma, cryptochidism, etc or treatment with chemotherapeutic drugs was excluded before inclusion into the study.
Methods: Study groups were comprised of 54 cases of MA, 52 cases of SCOS and 34 cases of HS. FISH with XY probes were carried out in addition to conventional chromosome analysis to find out sex chromosome aneuploidy. STS PCR analysis was carried out for Yq microdeletion studies. There were 50 normal fertile male served as control. For sertoli cell maturity status anti−mullerian hormone and for sertoli cell functional status inhibin B as well as seminal lactate were estimated by ELISA method. Serum heavy metals levels were evaluated in 90 cases. Later, in a subset of 37 idiopathic MA cases DNA microarray was carried out to find out any association with recurrent CNV/LOH.

Results: Underlying cause (Yq microdeletion and chromosomal abnormality) was detectable in 30 cases (21.4%) of PTF (13 sex chromosomal abnormality & 17 Yq microdeletions). When we dissected out in relation to subtypes we find different frequency of detectable causes. Detectable cause was found in 16 (11.4%) cases of SCOS, 8 (5.7%) cases of MA & 6 (4.3%) cases of HS. Heavy metal like manganese, lead and nickel were found consistently high (3−7X) in PTF than control (lead and nickel were 6−7X higher in MA than control). Microarray finding on idiopathic MA cases (37) showed recurrent CNVs of Yp11.31−p11.2 (15 cases with 3 copies), Yp11.2 (8 cases with 3 copies), Yq11.223 (6 cases with deletion), Yq11.23 (3 cases with deletion), Yq11.223−11.233 (3 cases with 3 copies), Xp11.23 (6 cases with 2 copies), Xq28 (4 cases with 3 copies), 14q32.33 (5 cases with 3 copies), 14q11.2 (3 cases with 3 copies), 7q11.1−11.21 (2 cases with 3 copies), 10q11.22 (2 cases with 3 copies), 16p11.2 (2 cases with 3 copies), 17p11.22 (2 cases with 3 copies) and 22q11.22 (2 cases with 3 copies).
Conclusion: Role of associated genes within CNVs in probable causation of maturation arrest will be discussed.

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SPERM TELOMERE LENGTH AND DNA INTEGRITY: ROLE IN IDIOPATHIC MALE INFERTILITY: IMPACTS OF LIFE STYLE INTERVENTIONS

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Introduction: Telomeres are highly conserved hexameric repeats which confer chromosome stability and maintain genomic integrity. Telomerase a reverse transcriptase maintain telomere length. PARP1 is a DNA repair enzyme recruited when there are double strand breaks in DNA. PARP−1 also plays a role in telomere maintenance. As telomeres are Guanine rich repeats, they are highly prone to oxidative damage. So, this study was planned to evaluate seminal oxidative stress, sperm DNA damage, sperm telomere length and telomerase activity in infertile men and also evaluate the effect of life style interventions (Yoga, Breathing exercises) on levels of telomere length and telomerase activity at (pre day 0, post yoga day 10 & 90).
Methods: The study included 33 infertile men and 30 controls. The average telomere length from the sperm DNA was measured using a quantitative Real Time PCR. Telomerase activity per cell was assessed by PCR ELISA. 8−Isoprostane and 8−Hydroxy−2−deoxy−Guaonosine levels were assessed by Cayman's ELISA kits. DFI was assessed by Sperm Chromatin Structure Assay (SCSA). DNA repair enzyme PARP1 expression was measured by q−PCR.
Results: The mean T/S ratio in patients was 0.742 ± 0.040 and controls 0.789 ± 0.066 (p=0.001). The mean Relative Telomerase activity per cell in patients was 25.48 ± 3.49 and controls 11.7 ± 1.71(p=0.148). 8−Isoprostane level in patients was 1154.14 ± 237.90 pg/ml and in controls 286.65 ± 80.35(p=0.004) pg/ml. The 8−OHdG level in patients was 48.36 ± 14.9 pg/ml and in controls 8.97 ± 4.64(p=0.136) pg/ml. The mean DNA Fragmentation Index (DFI %) in Patients was 38.86 ± 13.79 and in controls 27.4 ± 6.96 (p=0.044). PARP1 expression was significantly lower in patients compared to controls. With life style modifications telomere length showed no significant difference in 10 days (0.689±0.032), but significant increase after 3 months (0.851±0.051). Telomerase activity showed significant increase in 10 days (28.31± 1.150) and 90 days (34.31±1.067).
Conclusion: This study highlights oxidative DNA damage and preferentially telomere shortening in infertile men. Post life style interventions showed up regulation in telomere length and telomerase activity. Future of ART lies in selection of gametes with optimal telomere length and adoption of simple life style interventions may actually improve DNA health.

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INTEGRATIVE DNA METHYLATION AND GENE EXPRESSION ANALYSES IDENTIFIES DISCOIDIN DOMAIN RECEPTOR 1 (DDR1) ASSOCIATION WITH IDIOPATHIC NONOBSTRUCTIVE AZOOSPERMIA (NOA)

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(Presented By: Ranjith Ramasamy, MD)

Introduction: Spermatogenesis is a complex process that involves proliferation, differentiation, and cell adhesion. Spermatogenic failure or non−obstructive azoospermia (NOA) results from mechanisms involved are incompletely understood. DDR1 is a member of a small subfamily of receptor tyrosine kinases that is involved in adhesion, migration, proliferation, apoptosis, cell morphogenesis and differentiation. Since, DDR1 is expressed in human post−meiotic germ cells of testis, we hypothesized that abnormal DDR1 expression could be a possible mechanism that can compromise spermatogenesis in a subset of men with idiopathic NOA.
Methods: We used the high resolution Infinium 450K methylation array and compared fibrobalsts cultured from testicular biopsies of 19 NOA men and 4 fertile controls. Microarray data was analyzed using Minfi (R software package) utilizing subset−quantile within array normalization. We investigated the functional role of abnormal promoter DNA methylation for selected genes using mRNA expression by quantitative RT−real time PCR. Immunohistochemistry was used to confirm testicular expression and potential importance in spermatogenesis.

Results: Differentially methylated CpG sites (~20K) were identified using an F−Test ($p<0.05$) in the NOA samples. We identified 24 genes with the >30% difference in methylation within promoter region of men with NOA and fertile controls. Of the aberrantly methylated CpGs, 13 were hypomethylated and 11 were hypermethylated groups. From the top 11 hypermethylated genes, six genes (MRI1, DCAF12L1, TMEM95, CECR2, DDR1, NPHS2) were selected for validation since they were shown to be expressed in testis. Of the 6 genes validated with qPCR, DDR1 showed aberrant gene expression pattern. Four (21%) patients out of the 19 NOA men had lower expression levels (1.8x) of DDR1, whereas two (10.5%) men had higher expression levels (2.5x) of DDR1compared to fertile men ($p<0.05$). Immunohistochemical analysis suggests presence of DDR1 within cytoplasm of germ cells in fertile men and men with maturation arrest histology. DDR1 protein is absent in men with Sertoli−cell only or germ cell aplasia.
Conclusions: Aberrant expression of DDR1 is associated with NOA. The functional relevance of abnormal methylation of DDR1 to NOA warrants further investigation
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AFFECT OF OXIDATIVE STRESS AND SPERM DNA DAMAGE ON EARLY EVENTS OF CONCEPTION, INDICES OF EMBRYO GROWTH AND EMBRYO QUALITY IN COUPLES OPTING FOR IVF

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Introduction: Sperm genome plays a key role in maintaining reproductive potential. Impact of altered paternal genome is as important as that of maternal genome. However, while role of oocyte is being increasingly recognized, influence of male germ cells on conception is still not clear. The study investigates the association of reactive oxygen species (ROS) and sperm DNA damage on fertilization rate, cleavage rate, embryo quality and on pregnancy outcome in couples opting for in vitro fertilization (IVF).
Methods: In 278 infertile males opting for IVF and 124 fertile controls, ROS levels in semen was analyzed by chemiluminescence and sperm DNA damage was quantified by comet assay. Standard IVF protocol was adopted. Fertilization and cleavage rate, embryo quality and pregnancy outcome were followed and documented.
Results: ROS levels (32.41 RLU/sec/million) in non conceived group was significantly higher ($p=0.0325$) as compared to conceived group (22.19 RLU/sec/million). However, fertile controls had significantly lower ($p=0.0001$) ROS levels (16.73 RLU/sec/million) as compared to conceived group (22.19 RLU/sec/million). Increase in ROS was associated with decreased fertilization rate, cleavage rate and embryo quality in the conceived and the non conceived group. Sperm DNA fragmentation index (DFI) in conceived group (24.58) was significantly lower ($p=0.0001$) than non conceived group (34.17). Though DFI in conceived group (24.58) was significantly higher ($p=0.0002$) as compared to controls (18.95). Fertilization rate, cleavage rate and embryo quality had a negative correlation with DFI in non conceived group and conceived group. ROS levels and sperm DFI had no correlation with pregnancy outcome in both conceived and non conceived group. No correlation of sperm parameters was observed with any of the investigated parameters.
Discussion: Though ROS and sperm DFI adversely affect fertilization rate, cleavage rate and embryo quality, but in our study, ROS and DFI had no association with the pregnancy outcome probably due to selection of best quality of embryo for implantation during the IVF procedures. Thus ROS and sperm DFI have better diagnostic and prognostic capability to discriminate between fertile and infertile men. Considering the risk of childhood cancers, leukemias, and/or autism in children conceived by assisted conception, ROS and sperm DNA damage assessment should be included in workup of infertile males opting for assisted conception.

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DETECTING SPERM DNA FRAGMENTATION TO DISCRIMINATE BETWEEN FERTILE AND INFERTILE MEN

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(Presented By: Marta Cambi)

Introduction: Sperm DNA Fragmentation (sDF) is an anomaly of sperm genome consisting in single and double stranded DNA breaks. The impact of sDF on reproductive outcomes remains elusive due to the conflicting results of clinical studies. The ability of tests detecting sDF to predict the outcomes of reproduction is affected by many variables, including the sperm population where the damage is revealed.
Methods: Using TUNEL/PI, coupling the detection of sDF to the nuclear staining with propidium iodide, PI, our group unveiled two flow cytometric sperm populations that differ for PI staining (termed PI brighter and PI dimmer populations), for the amount of sDF and for cell viability. Indeed, PI dimmer sperm are all DNA fragmented and not viable. Conversely, PI brighter sperm are both fragmented and not fragmented and both viable and not viable. Based on this finding we reasoned that PI dimmer sperm have no chance to participate in fertilization, that the fraction of sDF really impacting on reproduction is that of PI brighter sperm and, within it, that of viable gametes. To verify this hypothesis, we set up a method able to detect sDF in viable spermatozoa by using a LIVE/DEAD fixable stain that labels dead cells permanently, thus remaining even after processing samples by TUNEL for sDF detection. Then we compared the levels of sDF as measured in total, PI brighter and live spermatozoa in 23 fertile and 22 infertile men.

Results: As expected, we found that sDF resulted increased ($p<0.05$) in infertile respect to fertile subjects, both in total (44.6±18.8 vs 35.2±13.6%) and PI brighter (33.2±15.8 vs 24.3±10.8%) and live sperm (25.1±19.3 vs 13.6±6.2%). However, the percentage increase in infertile vs fertile subjects was much greater for viable sperm (84.4%) respect to PI brighter sperm (36.4%) and total population (26.5%).
Conclusion: In conclusion, the ability of sDF to discriminate between fertile and infertile men, ameliorates considering PI brighter and above all viable sperm, respect to total sperm population.

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INFERTILITY, RECURRENT SPONTANEOUS ABORTIONS, CONGENITAL MALFORMATIONS AND CANCER: POINTS OF COMMON CAUSALITY

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(Presented By: Swetasmita Mishra, MSc)

Introduction: Infertility, recurrent spontaneous abortions (RSA), congenital malformation (CM) & cancer may have common underlying etiology. RSA is a common complication of pregnancy & the role of sperm factor has not been evaluated in idiopathic cases. The prevalence of CM is 2−3% worldwide; but role of paternal factors beyond karyotyping has not been studied. OS preferentially damages nucleohistone compartment of sperm genome. Telomeric DNA attrition disrupts homologous recombination, results in segregation errors, structural rearrangement and loss of DNA integrity and may have a role in infertility, RSA, CM and cancer. So, this study was planned to investigate sperm factors in these conditions. Non familial cases of childhood cancer (Retinoblastoma (Rb)) were enrolled who developed cancer by 1 yr of age & their father's sperm DNA integrity & OS were analysed.
Methods: 500 cases of idiopathic infertility, 86 couples with idiopathic RSA, 17 cases with CM, 41 cases of fathers of children with non familial cancer were enrolled for the study. Semen analysis, Seminal ROS was measured by chemiluminescence assay. 8−Isoprostane and 8−Hydroxy−2−deoxy−Guaonosine levels were assayed by Cayman's ELISA kit. DNA damage was assessed by SCSA. T/S ratio of sperm telomere length quantified by Q−PCR. Telomerase activity/ cell assessed by PCR ELISA.
Results: ROS levels (RLU/sec/million) were found to be higher than the controls in all the groups (infertile 47; RSA 38; CM 24.1; Retinoblastoma 36.086, Leukemia 24.69, controls< 22). The DFI% was also higher in the study groups (Infertility 31%; RSA 24%; CM 25%; Rb 43.50%) as compared to the controls (<21%). Telomere length was found to be significantly shorter in male partner of RSA or infertility cases. Levels of PARP were found to be significantly lower in these cases as compared to controls & explain for persistence of DNA damage. 8−Hydroxy−2−deoxyguanosine levels in sperm DNA were significantly higher in cases (infertile− 48.3pg/ml, Rb−165pg/ml) as compared to controls (8.9pg/ml).
Conclusion: Loss of sperm DNA integrity, accumulation of mutagenic bases, telomere shortening, chromosome aberrations, genome hyper mutability may be the underlying etiology of these disorders. Oxidative stress damages both mitochondrial and nuclear DNA. Evaluation of paternal factors must be included in diagnostic workup of couples having children with non familial cancer, CM, RSA and idiopathic infertility.

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MITOCHONDRIAL COPY NUMBER VARIATION: NO CORRELATION WITH SPERM DEFECTS: IMPLICATIONS IN ART

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Introduction: Mitochondrial DNA (mtDNA), the powerhouse of cell is not only source of ATP synthesis but also produces free radicals as byproduct. The number of mitochondria per cell type is highly variable depending on the cell's energetic demand. Although oogenesis is associated with a strong amplification of mtDNA copy numbers, spermatogenesis is associated with a drastic reduction in mtDNA content with maturation of sperm. Mature mammalian sperm are known to contain ~22−75 mitochondria. Few contradictory reports are available on mtDNA copy number amplification in poor quality sperm (impaired motility & morphological abnormality) and raise the concern of paternal mtDNA transmission due to defective oocyte filter with advancing age of couple opting for ART. Point mutations, deletions and the presence of a specific mtDNA haplogroup have been associated with poor sperm quality, but little attention has been paid to mtDNA copy number. Therefore, this study was planned to analyse mtDNA copy number in sperm with single defect, more than one defect and normal sperm.
Methods: For quantifying mtDNA, sperm DNA were isolated from mature spermatozoa of infertile men and fertile controls. MtDNA copy number was analysed by real time PCR in infertile men (n=66) and fertile controls (n=28) in order to compare the mtDNA content of normal and abnormal sperm. Of these 12 had single defects & 54 had defects in morphology & motility. The mtDNA/β−globin gene ratio was determined by realtime quantitative PCR.
Results: The average mtDNA copy number ratio was 1.11±0.209 in normal sperm (fertile controls) and 1.37±0.162 in abnormal sperm (cases with single & double defects). The ratio of patients with 2 abnormal criterion were 1.5±0.301 & with single abnormal criterion 1.25±0.105
Conclusion: Sperm with normal morphology & motility had 1.11 mtDNA copy number. This means that the majority of sperm are almost totally devoid of mtDNA, and that mature sperm probably do not contain any mtDNA at all. The mtDNA copy number was 1.37 in infertile men which was almost equal to normal sperm samples. There was no significant difference between abnormal and normal sperm mtDNA copy number. Thus there is no amplification of mtDNA copy number in poor quality sperm, thus no fear of risk of transmission of paternal mtDNA in ART.

ABSTRACTS

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THE ANALYSIS OF PATERNAL AGE ON INTRACYTOPLASMIC SPERM INJECTION OUTCOME

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(Presented By: Feng Jiang, MD)

Objective: In a retrospective study, advanced paternal age, fertilization rates and pregnancy rates after ICSI were compared. **Methods:** There were two age groups of men studied. Couples with male partners aged 60 years and over (group A) (n=27) with a mean age of 64±3 years were compared to couples with younger age−group male partners (group B) (n=57) with a mean age of 35±2 years. The control group of younger men was selected so that the women's age matched between the two groups.
Results: There was no significant difference in fertilization rate between the two groups (75.3 versus 82.4%). There was a significantly higher pregnancy rate in younger men (P<0.01). However, the long−term outcome of these pregnancies needs further investigation. Semen analysis showed significantly lower semen volume, sperm concentration and sperm morphology in group A versus group B (P<0.05), but these did not affect the fertilization rate.
Conclusion: It appears that paternal age has an effect on the pregnancy rate after ICSI.

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ACONITI LATERALIS PREPARATA RADIX IMPROVES SPERM MOTILITY THROUGH UP−REGULATION OF THE CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) PROTEIN IN CYCLOPHOSPHAMIDE−TREATED MALE MICE

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(Presented By: Seong Kyu Park, PhD)

Introduction: Male reproductive dysfunction associated with poor sperm motility and count is one of the important indicators for male infertility. Cyclic AMP response element modulator (CREM) plays a vital role for sperm development.
Methods: In this study, to examine the effect of Aconiti Lateralis Preparata Radix (ALR) on the sperm functions and the CREM expressions in mouse testis, C57BL/c male mice were divided into five groups; the normal group, cyclophosphamide(CP) only−treated group and ALR with CP (100, 500, 1000 mg/kg of ALR and 100 mg/kg of CP) treated group for five weeks. We performed real time PCR and western blot analysis for the examination of the CREM expression and analyzed sperm parameters. **Results:** In our results, sperm motility was markedly increased in 100, 500, 1000 mg/kg of ALR treated group than that of control group (15.11 ± 4.53, 13.07 ± 3.18 and 14.81 ± 2.16 vs. 3.63 ± 1.03%; $p < 0.001$, respectively). CERM expression levels were dose−dependently increased in ALR treated groups than that of control group.
Conclusion: In conclusion, our results suggest that ALR plays an important role in sperm motility and male infertility by up−regulation of the CREM expression.

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STUDY ON CONTRACEPTIVE EFFECT OF ETHANOL EXTRACTED JUSTICIA GENDARUSSA BURM.F. LEAVES IN FERTILE MEN: PHASE II CLINICAL TRIAL

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(Presented By: Dyan Pramesti, MD, Master)

Introduction and Objectives: Previous laboratory and clinical research suggest that Gendarussa has a contraceptive effect by preventing fertilization without effecting sperm macroscopic and microscopic parameters. It may decrease human sperm hyaluronidase activity, and sperm proteins of weights 38 kDa and 41.5 kDa were missing in the treatment group. A similar pattern of missing proteins has been found in infertile males. We have carried out a phase 2 clinical trial with larger sample size and short duration of Gendarussa administration. To quantify the reduction of sperm hyaluronidase activity and the disappearance of proteins at 38 kDa and 41.5 kDa; to determine pregnancy rate; to monitor the safety and reversibility of Gendarussa.
Methods: 70% ethanol extract of alkaloid−free Justicia gendarussa leaves was used. The subjects were 350 healthy fertile men age 21−40 years, normozoospermia, had at least one child and fulfilled other inclusion criteria. Single blind non−randomized method was undertaken. Group one (186 men) took 450 mg of Gendarussa capsules daily for 30 days; group two (164 men) took placebos and were instructed to use condoms. Semen analysis, hyaluronidase activity, sperm protein profile were examined before, during and after treatment. Subjects and their spouses were told to discontinue any contraception except Gendarussa or condoms. Group one was directed to have sexual intercourse three times during ovulation phase, after taking 20 capsules. The Ovulation phase was assessed individually for each couple. Spouses were asked to return after intercourse for post coital testing the next morning. Men in group two (placebo) were told to continue using condoms.
Results: Reduction of hyaluronidase activity by 5.81% and 6.47% after 15 and 30 days respectively and disappearance of band 38kDa and 41,5kDa of sperm protein after 5 days treatment, in group one. One pregnancy was found (1/186 = 0.54%), with strong suspicion caused by Gendarussa failure or possibly by not taking the medicine as directed. Hyaluronidase activity and sperm protein band 38kDa and 41,5kDa were found to be normal 30 days after stopping medication.
Conclusions: 70% ethanolic extract of alkaloid−free Justicia gendarussa leaves is an effective male oral contraceptive method that is reversible and had no serious adverse effects. Further study is needed to determine its mechanism of action, but data strongly suggest that it acts as a contraceptive by preventing fertilization.

ABSTRACTS

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ADVERSE EFFECTS OF CLOMIPHENE CITRATE IN INFERTILE MEN

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(Presented By: Mary Samplaski, MD)

Introduction: Clomiphene citrate (CC) is a selective estrogen receptor modulator, which has been used for the empiric treatment of male infertility with mixed results. We sought to determine the adverse effects of CC use in infertile men.
Methods: 85 men presenting for fertility evaluation from 2008−2013 were started on empiric CC. Data were analyzed for semen and hormonal parameters prior to starting CC, and then at 1 and 3m. At follow up men were queried about side effects experienced on CC.
Results: The most common starting dose of CC was 25 mg PO daily. 7 men had aromatase inhibitors started for rising serum estradiol. Side effects were reported in 18 men (121%), including: Dizziness (2), increased aggressivity or temper (2), gynecomastia (1), increased libido (8), decreased libido (3), bad taste in mouth (1), and back pain (1). 40 men (47%) had no improvement in total motile count (TMC) after CC. Of these 13 (32.5%) were azoospermic at the start and end of treatment. The remaining 27 (67.5%) had worsening of their semen parameters. 12 men had a decrease in TMC at 1m: 18.9±21.9 M to 10.7±11.2 M; mean decrease of 8.2±11.8 M, range 0.1−41.2 M; 5 men had a decrease in TMC >5M. 6 men discontinued CC at 1m due to semen parameters. 22 men had a decrease in TMC at 3m: 13.2±21.4 M to 7.8±11.7 M; mean decrease of 5.4±16 M. 7 men had a decrease in TMC >5M. Of the 22 men that had a decrease in TMC at 3m, 7 had a decrease at 1m but chose to continue CC. There were 2 men who had a substantial decrease in TMC on CC. 1 had a decrease of 41.4M at 1m, but related a possible incomplete collection. 1 had a decrease of 65.2M at 3m, however the samples were collected at different labs and motility was the primary difference. For the 7 men with a decrease in TMC >5M at 3m, hormonal parameters were as follows: The mean baseline FSH was 4.1±2.3 IU/L, the mean increase in FSH at 1m was 4.5±1.9, and at 3m 4.5±2.3. The mean baseline testosterone (T) was 8.4±4.0 nmol/L, the mean increase in T at 1m was 16.9±9.3, and at 3m 17.9±11.6. These hormonal changes were not different from those in men with a positive response to CC.
Conclusions: CC is well tolerated in men, with the most common adverse effects being increased libido and mood changes. There was a group of men that had worsening of their semen parameters, although these decreases were usually small. There were no clear predictors for these men.

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COCAINE USE IN THE INFERTILE MALE POPULATION: EFFECTS ON SEMEN AND HORMONAL PARAMETERS

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Introduction: The United States is the world's largest consumer of cocaine. Cocaine is commonly used in upper−middle class communities, the same group of men who often present for male fertility evaluation. We sought to evaluate the incidence of cocaine use in the infertile male population and if cocaine use is associated with changes in semen parameters.
Methods: Men presenting for a fertility evaluation from 2008−2012 reporting using cocaine were identified via a prospectively collected database. Data were analyzed for semen parameters.
Results: 39/4400 (0.8%) men reported using cocaine at presentation. Concurrent reported drug use was reported in 90% of the men and included: marijuana (32), ecstacy (9), LSD (2), heroin (1) and anabolic steroids for bodybuilding (3). 4 men reported using cocaine monthly, the rest reported using cocaine every 3 months or less. 5 couples had prior children and 4 couples reported therapeutic abortions. One man was a longstanding diabetic, with retrograde ejaculation. After trying cocaine for the first time he had his first antegrade ejaculation in many years, with a total sperm count (TSC) of 131 M. There were a number of other clear causes for infertility including 5 men taking cocaine who were seen for vasectomy reversals, 4 men who had oncologic therapies rendering them azoospermic and 2 men who were seen for biopsy−proven early maturation arrest. After excluding these men and those using anabolic steroids, 16 men had semen analyses available for analyses. For these men, the mean semen parameters were: ejaculate volume 4.48 ± 2.64 mL; sperm concentration 13.37 ± 13.79 M/mL; motility 22 ± 15.7 %; TSC 109.83 ± 133.66 M.
Conclusions: There are very few reports on the use of cocaine among men presenting for a fertility investigation: this report indicates that cocaine use in our centre is rare among men presenting for an infertility investigation and does indicate that most of the infertile men on cocaine have relatively preserved semen parameters.

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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 Srini, DNB, Fellowship)

Objective: To predict the accuracy of sperm retrieval by using such non−invasive markers in order to avoid the morbidities and complications of surgery.
Methods: Prospective, non−randomized cohort study. Andrology unit in a Tertiary Fertility Centre, India. 100 consecutive patients diagnosed to have non obstructive azoospermia between January 2009 and December 2010 and undergoing testicular sperm extraction (TESE). Patients with azoospermia scheduled for TESE: Serum Inhibin B and epididymal head size were measured. The biopsy report after TESE was recorded. 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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INFLUENCE OF AN AROMATASE INHIBITOR ON SEXUAL FUNCTION IN MEN WITH NON−MOSAIC KLINEFELTER'S SYNDROME

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(Presented By: Sotirios Koukos)

Introduction: We evaluated the role of anastrazole, an aromatase inhibitor, in the sexual function of men with non−mosaic Klinefelter's syndrome.
Methods: Twenty one men with non−mosaic Klinefelter's syndrome were divided into two groups A and B. Men of group A (n=13) received daily anastrazole (1 mg daily) for 12 weeks. Men of group B (n=8) did not receive any pharmaceutical treatment for a period of 12 weeks. There was not significant difference in the mean age of the participants of group A and B. The IIEF−5 questionnaire (Int J Impot Res,199;11;319) was completed by each participant of groups A and B at the beginning of the study and 12 weeks later (end of the study). Peripheral serum levels of testosterone were recorded, within each group, at the beginning of the study and at the end of the study. Within each group, the mean value of IIEF−5 outcome or testosterone at the beginning of the study and at the end of the study were compared using Wilcoxon test for paired observations. A probability P smaller than 0.05 was considered to be statistically significant.
Results: Within group A, mean IIEF−5 outcome or mean testosterone value was significantly larger at the end of the study than in the beginning of the study. In contrast, within group B, there was not significant difference in the mean IIEF−5 outcome or in the mean testosterone value between the beginning of the study and the end of the study.
Conclusion: It appears that anastrazole treatment increasing serum testosterone profiles improves sexual function in men with non−mosaic Klinefelter's syndrome. Additionally the increase in serum testosterone in men of group A may improve each individual psychology and self confidence with an overall positive effect on sexual function.

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INHIBITORY PROPERTIES OF POMOGRANATE JUICE ON HUMAN CORPUS CAVERNOSUM: EXPRESSION OF NOS ISOFORMS AND PDE5A1 ENZYMES

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(Presented By: Serap Gur, PhD)

Introduction and Objectives: Pomegranate juice (POM Wonderful, Los Angeles, CA) may benefit the erectile process. Molecular characterization and in vitro confirmation of its effect are lacking. The present study evaluated the action of POM on human corpus cavernosum (HCC) smooth muscle.
Methods: HCC tissues from patients (age:47−75, n=9), undergoing prosthesis implantation were placed in organ baths. After phenylephrine (PE, 10 μM) contraction, the relaxant effect of POM with or without several inhibitory and stimulatory agents were evaluated. Ex vivo organ culture of HCC was performed and cells were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture (DMEM)−F12 and kept at 37°C and 5% CO2. Cells from early passage (p 3−5) were treated with 10 μl/ml (v:v) of POM and mRNA was collected. The expression of neuronal NO synthase (nNOS), endothelial (e)NOS, and phosphodiesterase (PDE)−5A was assessed by RT−PCR analyses.
Results: Our study demonstrated that POM in HCC induced marked relaxation (maximum response: 97.0±3.1%), which was not inhibited by nitric oxide (NO) synthase inhibitor L−NAME (100μM) and the soluble guanylyl cyclase inhibitor ODQ (10μM). POM potentiated EFS, but not addition of ACh (10μM), sildenafil (10μM) or sodium nitroprusside (SNP 0,1μM). The expression of nNOS was 7.2 ± 3.2 fold higher in POM−treated cells compared to controls ($p<0.0121$). There was no significant change in eNOS ($p<0.2715$) and PDE−5A ($p<0.09$) compared to controls.
Conclusions: POM induces marked relaxation of HCC and its effect is not by activation of the NO/cGMP pathway. Data from RT−PCR indicates that nNOS is the most robust response. POM may synergize with the neuronal reflex activated by nNOS to signal downstream relaxation, by bypassing NO/cGMP and PDE5systems. Hence, this food additive may help men with ED who do not respond fully to oral PDE5 inhibitor.

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EVALUATION OF THE CHRONIC TREATMENT WITH RESVERATROL ON THE METABOLIC AND REPRODUCTIVE PARAMETERS OF YOUNG ADULT RATS WITH TYPE 1 DIABETES INDUCED BY STREPTOZOTOCIN IN THE PREPUBERTY

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(Presented By: Joana N.Simas)

ABSTRACTS

Introduction: Diabetes Mellitus is a metabolic disorder of multiple etiology and epidemic proportions. Its pathogenesis unleashes the progression of a variety of complications, among which reproductive alterations. Resveratrol (RES), a fitoalexin found in several plants, constitutes a powerful antioxidant that also presents antidiabetic activity. Recent report has suggested that RES can improve spermatogenic parameters that are altered due to testicular ischemia. Our goal is to assess the following trilogy: type 1 Diabetes (DM1), male reproduction and a possible benefit promoted by RES.
Methods: Eighty−four prepubertal male Wistar rats were used to compose 7 groups: absolute control (C); sham control (SC, treated with Carboximethilcelulose, which is RES vehicle); RES−treated (R); diabetic (D); diabetic insulin−treated (DI); diabetic RES−treated (DR), diabetic insulin− and RES−treated (DIR). DM1 was induced by a single intraperitoneal injection of streptozotocin (65 mg/kg) on the 30th day postpartum (dpp). Animals of DR, DIR and R groups received a daily dose of RES (150mg/day by gavage route) for 42 consecutive days (from the 33 dpp on). DI and DIR rats received daily subcutaneous injections of insulin (1U/100g bw) from the 5th day after the DM1 induction. An oral glucose solution was offered on the 1st (2.5%) and on the 2nd day (5.00 %) after the detection of DM1 to avoid abrupt hypoglycemia.
Results: The blood glucose measurement (BGM) of all rats was obtained at 4 different time−points: before the STZ treatment, on the 3rd day post treatment, at 45 dpp (peripuberty) and at 64 dpp (postpuberty). At 75 dpp (young adult phase) the rats were submitted to euthanasia for biometric and morphometric testicular analyses and spermatic evaluation. The BGM in the D group was significantly higher than in the DR, DI and DIR groups. The age of preputial separation was delayed in the induced−groups. The D group presented significantly reduced body weight when compared to the DR and DIR groups, as well as reduced relative testicular weight when compared to the DR and DI groups. Rats of the DR and DIR groups showed an increased frequency of morphologically normal sperms in the epididymal cauda and an improvement in the sperm mitochondrial activity when compared to the D and DI groups.
Conclusion: These results indicate that RES improve both glycemia and sperm quality parameters in diabetic rats. Additional metabolic analysis, sex hormone dosages and supplementary reproductive evaluations are being carried out.

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AN OBJECTIVE EVALUATION OF VIBERECT® (MALE VIBRATOR DEVICE) IN INDUCING FUNCTIONAL ERECTION IN COMPARISON TO INTRACAVERNOSAL VASOACTIVE INJECTION USING PENILE DUPLEX DOPPLER ULTRASOUND BLOOD FLOW ANALYSIS

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(Presented By: Suresh Sikka, PhD)

Introduction and Objective: Viberect® is a new FDA−cleared medical vibrator device that stimulates genital afferent nerves and induces penile erection. The degree and quality of penile rigidity induced by Viberect® has variable response and depends upon many factors. An objective evaluation of functionality of such device is needed. To compare erection rigidity and penile blood flow induced by Viberect® versus intracavernosal injection (ICI) of a vasoactive agent in patients undergoing color duplex Doppler ultrasound (CDDU) evaluation.
Methods: One hundred five ED/Peyronie's patients attending our Andrology & sexual dysfunction clinic during 2011−2013 consented to receive instructions and correctly use the Viberect® prior to undergoing penile CDDU. Viberect® stimulation was performed by patients at 70−100 Hz for 6−10 minutes and CDDU performed as per our standard protocol (JSM, 2013). After the penis becomes flaccid, an ICI (7−15mcg prostaglandin E1, PGE1) was administered and CDDU repeated by the same sonographer under similar environment and visual sexual stimulation (VSS) settings.
Results: Thirty three men (called "positive−responders"to Viberect®) showed >60% rigidity and 55 cm/sec mean peak systolic velocity (PSV) with Viberect® compared to >65% rigidity ($p>0.05$) and 70 cm/sec mean PSV (2−tailed paired t−test value of $p<0.05$) with PGE1. Forty five patients (called "borderline−responders") showed 36% mean rigidity and 44cm/sec PSV with Viberect® compared to 58% rigidity and 66cm/sec PSV with PGE1 ($p< 0.002$). Only 15 patients (called "non−responders") showed poor erection response with Viberect® (mean 15% rigidity and 29cm/sec PSV) compared to mean 56% rigidity and 59cm/sec PSV with PGE1 ($p<0.001$). Twelve patients could not complete Viberect® stimulation due to impending ejaculation. No complaints or adverse events were reported with Viberect®. Thus, Viberect® induced good blood flow and rigid erection response almost similar to ICI in "positive−responders". Many "borderline and negative responders"had high anxiety/environmental issues using this vibrator in clinical setting.
Conclusions: This study suggests that Viberect® that stimulates bulbocavernous and pudendo−cavernous reflex is safe, convenient, well−tolerated modality for inducing erection. Randomized prospective multicenter trials using standardized CDDU should be performed to further validate the concept of stimulating these reflexes with such vibrators for ED diagnosis and treatment.

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SURVEY OF THE RECOGNITION OF CIRCUMCISION

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(Presented By: JoonYong Kim)

Objective: Historically, circumcision is a very old surgical procedure but there are lots of debates and the regional, religious and cultural differences are shown regarding frequency, timing, reason, etc. Since the 1950s in Korea the frequency of circumcision has increased until recent years that it is stagnant or declining. We report awareness of Korean men about circumcision.
Method: 91 people at the age of 20 to 59 were participated in questionnaire with 16 questions about timing of surgery, medical professionals, motivation, surgical outcome, side effects, changes in sexual function, etc.

Result: The average age was 40.1 years old. The timing of surgery is 20s (46%), grades 1−3 in elementary school (14.6%), grades 4−6 in elementary school (12.4%) and preferred timing of surgery is grades 4−6 in elementary school (18.9%), high school (18.9%). Medical department is urology (42.7%), don't know (22.5%), army surgeon (6.7%). Surgical motivation is hygienic reason (33.7%), parents' recommendation (30.8%). Side effect is unobserved (86.6%) and complaints about surgery is insignificant (56.5%), not enough skin (19.6%). Sufficiency of penis skin at flaccid state after surgery is full exposure of glans (78.4%), coverage of partial glans (17%). Desired sufficiency of penis skin is fully exposed glans with folded skin (69%), partial (half) glans covered (19%). Change of Penile size is unobserved (44.4%), don't know (35.6%). Change in sensation is don't know (46%), unobserved (24.7%). Expectation after surgery is hygienic improvement (33.1%), prevention of sexual transmitted disease (24.1%). Necessity of circumcision is necessary (64.8%), highly necessary (14.8%) and appropriate medical department is urology (96.7%). Changes in sexual function after surgery is not observed (78.4%), improved (3.9%), worse (3.9%).
Conclusion: High incidence of circumcision in age 20s is because of the military service, etc. and the top motivation is hygienic reason but many chose to circumcise due to parents' recommendation and trend. This means that most decisions were passive and conventional. From the complaint that there is not enough penile skin after surgery and tightness at erection and the fact that many preferred sufficient penile skin, remaining enough skin after surgery should be considered. Expectation of surgical outcome is hygienic improvement as well as prevention of disease. The necessity and positive recognition of circumcision was relatively high and in reality.

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IMPACT OF LIFE STYLE INTERVENTIONS ON MARKERS OF CELLULAR AGING

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(Presented By: Shiv Basant Kumar, MSc)

Introduction: A hectic life style, psychological stress, increased fast food intake, increased electromagnetic radicals exposure leads to exposure to free radical. Hence this study was planned to evaluate role of life style intervention on various stress markers such as Cortisol, 8−hydroxy−2'deoxyguanosine (8−OHdG) and Reactive Oxygen Species (ROS) and inflammatory markers like Telomere length and Telomerase activity.
Objective: To evaluate effects of life style interventions (yoga) on markers of cellular aging and free radical levels. The telomerase activity and Telomere length which maintains chromosomal stability were assessed.
Methods: 50 healthy volunteers enrolled in IHC. Information was obtained about their lifestyle using a questionnaire about their life, such as food choices, habits and socioeconomic status. Venous blood samples were collected. Stress markers such as Plasma Cortisol, 8−OHdG and blood ROS levels were measured. We also assessed the telomerase level and telomere length.
Results: There was a significant reduction in various markers of oxidative stress in subjects and an increase in telomerase level at day 0 vs. day 10. Telomere length did not show any significant change. The mean Cortisol levels were significantly lower (P = 0.0072) in the subjects (pre yoga) (118.83 ± 30.58) ng/mL compared to 10days after practicing yoga (96.32 ± 36.06) ng/mL, while ROS level decreased from baseline to day 10 (1215.069 ± 0.88, 1020.81 ± 0.79 RLU/min/104 Neutrophils; p=0.024). Although 8−OHdG levels were reduced (10268.23± 3349.71 vs. 9367.57 ± 2709.58pg/mL) after yoga intervention, the difference was not statistically significant (p=0.459). Telomerase levels were elevated post intervention[(0.59 (0.114 − 2.043)IU/Cell Vs 2.40 (0.568 − 5.448)IU/Cell] but telomere length did not show any change.
Conclusions: This short time yoga−based lifestyle intervention reduced the markers of stress even in 10 days in the general population. We are following these cases up to 3 months but this study is ongoing. Decline in free radical levels may actively prevent several diseases in which oxidative stress is one of the chief causative factors. Telomerase level upregulation is key factor in maintenance of Telomere length which maintains genomic integrity. This yoga based life style interventions may be recommended as therapeutic in decreasing oxidative stress and oxidative DNA damage.

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EXCESSIVE EXTRACELLULAR ATP FORMATION BY MALIGNANT CELL−DERIVED PROSTASOMES DUE TO DOWN-REGULATED ATPASE ACTIVITY

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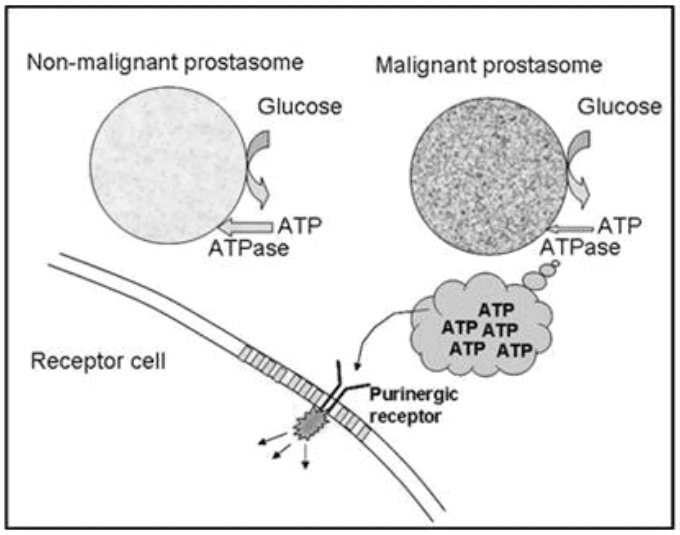
Dep. of Med. Science
(Presented By: K. Göran Ronquist, PhD)

Introduction and Objectives: Cancer with all its complexity means influences by not only intracellular genetic and epigenetic changes but also by stromal cells, local extracellular matrix and metabolic courses of events in the microenvironment. Prostasomes are small extracellular membrane vesicles with an endosomal origin that are released by prostate acinar cells into the extracellular environment. We wanted to investigate the overall energy metabolic capability of prostate cancer cell−derived prostasomes in comparison with their non−malignant counterparts in terms of net ATP gain after incubation with proper substrates.
Methods: Prostasomes were harvested from the growth medium of cancer metastatic PC3 cells and subjected to differential centrifugation steps including preparative ultracentrifugation, filtration through a 0.20 μm filter and sucrose gradient ultracentrifugation. Human seminal (non−malignant) prostasomes were subjected to a similar purification procedure where the filtration was replaced by gel chromatography. Prostasomes were incubated with and without glucose in presence of ADP and ATP was determined by a luciferin/luciferase method.
Results: PC3 cell−derived prostasomes displayed a 10−fold lower ATPase activity compared with seminal prostasomes. Both types of prostasomes were able to form ATP in about equal amounts by glycolysis in addition to adenylate kinase−catalyzed formation of ATP.
Conclusions: The net ATP gain of PC3 cell−derived prostasomes was high due to their low ATPase activity and this ATP may be at disposal of purinergic receptors and/or protein kinases in the cancer cell microenvironment.



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IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ON SUSCEPTIBILITY OF GALECTIN−3 TO CLEAVAGE BY PROSTATE SPECIFIC ANTIGEN (PSA)

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Introduction: Galectin−3 is a multivalent, carbohydrate−binding protein involved in cell adhesion, immunomodulation, and cancer progression, including prostate cancer. In the human male reproductive tract, galectin−3 function is regulated, in part, by proteolytic processing by PSA, which abrogates the ability of galectin−3 to oligomerize. Significantly, proteolytic cleavage of galectin−3 is associated with prostate cancer progression. The SNPs rs4644 and rs4652 generate proline (P)−to−histidine (H) and threonine (T)−to−P polymorphisms at amino acids 64 and 98, respectively, in galectin−3. Thus, these SNPs create four possible galectin−3 variants in humans (P64T98, P64P98, H64P98, H64T98).
Methods: To investigate the effects of galectin−3 allelic variation on susceptibility to PSA proteolytic cleavage, in vitro cleavage assays compared PSA proteolysis of each galectin−3 variant individually to emulate homozygous phenotypes and in pair−wise combination to emulate heterozygous phenotypes. Immunoblot analysis of galectin−3 cleavage products indicated that the galectin−3 H64 variants were up to 3.5−fold more susceptible to cleavage by PSA than were the P64 variants. The pair−wise combinations of galectin−3 P64T98/H64P98 and galectin−3 P64P98/H64P98 were cleaved by PSA with at least two−fold greater efficiency than was galectin−3 P64T98/P64P98. Moreover, the H64 variants exhibited an additional cleavage product not observed for the P64 variants, indicating that galectin−3 H64P98 and H64T98 contain a PSA cleavage site that is not present in galectin−3 P64T98 or P64P98.
Results: Immunoblot analysis identified a nearly identical galectin−3 cleavage pattern in prostate tumor lysates and PSA−cleaved galectin−3 variant samples, but not in matrix metalloprotease (MMP)−2 or MMP−9 cleaved galectin−3 samples. These results suggest that PSA is involved in cleaving galectin−3 in the prostate tumor microenvironment. Immediate future studies will identify the additional cleavage site in galectin−3 H64 variants, will determine whether there are any differences in secondary or tertiary structure between the four galectin−3 variants, and will evaluate the ability of the galectin−3 variants to form homo− and hetero−oligomers.
Conclusion: Overall, our results indicate that the galectin−3 genotype determines the susceptibility of galectin−3 to proteolytic cleavage by PSA and implicate galectin−3 genetic polymorphism as an etiological factor in prostate cancer progression.

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FLAGELLAR BIOGENESIS: A POTENTIAL LINK BETWEEN MFN2 AND MNS1

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(Presented By: Melissa Vadnais, VMD, PhD)

Introduction: MNS1 is a recently characterized protein that is abundantly expressed in post−meiotic spermatids and is required for proper flagellar formation. To explore the possible functions of MNS1, we performed a BLAST search and identified the conserved domain pfam13868, exemplified by trichoplein. This protein interacts with mitofusin 2 (MFN2), a protein that participates in regulating mitochondrial associations to subcellular organelles. We hypothesized that an association between MFN2 and MNS1 in the sperm is involved in flagellar biogenesis and function.
Methods: In the studies reported here, MFN2 was found in murine reproductive and somatic tissues high in ciliary content, and MNS1 was present as two closely migrating bands in reproductive tissues. Similar to Mns1, Mfn2 was expressed in the testis as detected by RT−PCR. In addition, Mfn2 and Mns1 decreased in expression from pachytene spermatocytes to condensing spermatids as assessed by quantitative RT−PCR. Co−immunoprecipitation demonstrated an association between MFN2 and MNS1 in spermatogenic cells. Indirect immunofluorescence indicated that MFN2 and MNS1 co−localized to the sperm flagellum in freshly collected cauda epididymal sperm. MFN2 associated with the midpiece while MNS1 was present throughout the sperm tail in caput and cauda epididymal sperm.
Results: In spermatogenic cells, MFN2 was seen in the mitochondria, and MNS1 was present throughout the cytoplasm. MFN2 and MNS1 were present in detergent−resistant structures of the sperm.
Conclusion: These results demonstrate that these proteins are present in spermatogenic cells and are an integral part of the sperm flagellum, indicating they may play a role in flagellar biogenesis and/or function.
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ABSTRACTS

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ADENYLATE KINASE 8, ADENINE NUCLEOTIDE METABOLISM, AND A ROLE FOR AMP IN MODULATING FLAGELLAR WAVEFORMS IN MOUSE SPERM

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Introduction: While most ATP, the main energy source driving sperm motility, is derived from glycolysis and oxidative phosphorylation, the metabolic demands of the cell require the efficient use of power stored in high−energy phosphate bonds. In times of high energy consumption, adenylate kinase (AK) scavenges one ATP molecule by transphosphorylation of two molecules of ADP, simultaneously yielding one molecule of AMP as a byproduct.
Methods: We previously demonstrated that AK1 and AK2 are present in outer dense fibers and mitochondrial sheath of the mouse sperm tail. Here we show that another AK, AK8, is present in third flagellar compartment, the axoneme. As a functional test of AK, either ATP or ADP supported motility in detergent−modeled mouse cauda epididymal sperm. While ATP or ADP fueled motility, the resultant flagellar waveforms were qualitatively different. Motility driven by ATP was rapid but restricted to the distal region of the sperm tail whereas ADP produced slower and more fluid waves that propagated down the full flagellum.
Results: Characterization of wave patterns by tracing and superimposing the images of the flagella, quantifying the differences using digital image analysis, and computer−assisted sperm analysis revealed differences in the amplitude, the periodicity, and propagation of the waves between detergent−modeled sperm treated with either ATP or ADP.
Conclusion: Surprisingly, addition of AMP to the incubation medium containing ATP resulted in a pattern of sperm motility similar to that supported by ADP alone. These results extended the known regulators of sperm motility to include AMP, which may be operating through an AMP−activated protein kinase. Grant support: NIH grants R01HD051999, R01HD057144, T32HD007305, and P30ES013508

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VARICOCELECTOMY: CLINICAL IMPLICATIONS AND PROGNOSIS IN MANAGEMENT OF INFERTILITY.

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(Presented By: Monis Bilal Shamsi, MSc, PhD)

Introduction: Varicocele is implicated as a major cause of testicular dysfunction in large number of infertile men. The varicocele results in decline of testicular function due to testicular hypoxia and hyperthermia which at molecular level alters normal production and maintenance of sperm. Increased levels of reactive oxygen species (ROS) and reduced total antioxidant capacity (TAC) in men with varicocele suggested that sperm dysfunction may be in part related to the sperm DNA damage induced by oxidative stress. The study was planned to assess the efficacy of varicocelectomy by comparing the OS and sperm DNA damage pre varicocelectomy and one and six months post varicocelectomy.
Methods: Forty three patient with clinical varicocele and 34 normozoospermic healthy controls were enrolled in study. Sperm DNA damage was assessed by Comet assay and ROS by luminol induced chemiluminescence. TAC was quantified by commercially available kit. Analysis was done pre varicocelectomy and one and six months post varicocelectomy.
Results: A remarked improvement in sperm DNA quality and reduced oxidative stress levels was observed 6 months post varicocelectomy (Table 1).
Conclusion: Varicocele is commonest surgically reversible cause of male infertility. Antioxidant supplementation and varicocelectomy are most common thereupatic approach in treatment of varicocele. Varicocele patients have high ROS levels in semen than fertile controls. Prolonged exposure to ROS would lead to irreversible sperm DNA damage consequently resulting in decreased fertility. The high ROS and low TAC levels showed significant improvement one month post varicocelectomy but DNA integrity improved significantly only after 6 months. Therefore we emphasize that though oxidative stress may significantly decline immediately following varicocelectomy, DNA damage takes longer to revert to normal, since genomic integrity is an important prerequisite for fertilization and embryogenesis and birth of healthy offspring. Such men with varicocele should attempt pregnancy only after 6 month of varicocelectomy.

	Healthy Control	Patients		
		Pre Varicocelectomy	Post Varicocelectomy (One month)	Post Varicocelectomy (Six months)
ROS (RLU/sec/million)	15.37 ± 4.98	159.42 ± 27.18	98.87 ± 73.45	25.27 ± 9.36
TAC (mM)	6.7 ± 2.9	1.7 ± 0.5	2.9 ± 1.5	4.3 ± 0.8
% sperm with DNA damage	14.2 ± 7.36	38.29 ± 10.43	32.43 ± 9.54	20.56 ± 6.43

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THE CATSPER CALCIUM CHANNEL IN HUMAN SPERMATOZOA: RELATION WITH MOTILITY AND INVOLVEMENT IN PROGESTERONE−INDUCED ACROSOME REACTION

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(Presented By: Lara Tamburrino, PhD)

Introduction: KO mice for any of the CatSper family genes, fail to acquire hyperactivated motility (HA) and are infertile. Less clear is the role of CatSper in human sperm HA/activated motility and in asthenospermia. Few men with CatSper mutations have been described but sperm motility and the ability to achieve HA has not been well established. CatSper has been shown to mediate progesterone (P)−induced Ca2+ influx in human sperm but whether it is involved in the acrosome reaction (AR) inducing effect of the steroid has not been established.
Methods: We evaluated the effects of two Catsper inhibitors, NNC55−0396 (NNC, 10 and 20 μM) and Mibefreadil (Mib, 30 and 40 μM), on human sperm motility parameters and P−induced AR. Catsper1 protein expression was evaluated in unselected and swim up selected sperm samples and in spermatozoa of normo− and astheno−spermic subjects. Semen sample kinematic parameters were analyzed by CASA. A fluorescent labelled lectin was used to evaluate P−induced AR in live spermatozoa. CatSper1 protein expression was determined by western blot and by flow cytometry. Intracellular calcium concentrations ([Ca2+]i) were evaluated by a spectrofluorimetric method following sperm loading with the calcium sensitive probe fura 2/AM.

Results: CatSper1 protein was localized in the tail and its expression was found highly increased after swim up selection both by western blot and by evaluation of the percentage of spermatozoa expressing the protein by flow cytometry (27.2±9.0% in unselected vs 52.7±15.8% in selected, n=7, p<0.01). Basal and P−stimulated [Ca2+]i were significantly higher in swim up selected sperm respect to 40% PureSperm selected (n=8, p<0.05). Basal [Ca2+]i evaluated in 40% PureSperm selected spermatozoa was significantly related to progressive motility of the samples (r=0.71, p=0.04, p=0.01, n=8). CatSper1 expression was decreased in astheno− (n=10) respect to normo−spermic (n=9) men (p<0.01) and was positively related the percentage of sperm with progressive motility (r=0.59, n=19, p=0.007).
Conclusion: NNC and Mib significantly reduced sperm progressive motility and several kinematic parameters but did not affect the HA. Mib showed a significant effect on sperm viability. P−stimulated AR was significantly reduced by both inhibitors (p<0.05). Our results indicate that, in human spermatozoa, CatSper channel expression and function are associated to progressive motility and may be involved in the pathogenesis of asthenozoospermia and in the AR process.

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SPERM'S MEMBRANE CHARGE: AN INTERESTING BIOMARKER FOR NON−INVASIVE METHOD OF SPERM SELECTION

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Introduction and Objective: The electrostatic property of sperm was first introduced in 1991, since then only a few research groups have used this concept for the selection of better sperm. The sperm head is covered by a negatively charged coating (20−60 nm thick), to facilitate the interaction with its extracellular environment. Mature sperm possess an electric charge of −16 to –20 mV. The negatively charged glycocalyx adjacent to the sperm plasma membrane helps to prevent sperm from self−agglutination and non−specific binding with the genital tract epithelium during its transport and storage. In a normal and matured sperm, the membrane glycocalyx are rich with sialic acid residues. High levels of sialic acid residue in the sperm's membrane increases its net negative charge, for its role during capacitation, and its possible participation in the formation of binding bridges between sperm membrane and ovum. The aim of this study is to determine the association between sperm's membrane charge and ART outcomes.
Methods: Under the electric field, the percentage of sperm with positively (PCS), negatively (NCS) and neutrally charged sperm were determined in the ejaculate of 81 patients undergoing IVF treatment and associated with their ART outcomes.
Results: The percentage of NCS in the ejaculate was positively associated with fertilization rate (r2 = 0.381, p = 0.050), embryos that developed to blastocyst (r2 = 0.315, p = 0.010) and inversely associated with the percentage of arrested embryos (r2 = −0.264, p = 0.032). However, there was no significant correlation between the sperm's charge and ICSI fertilization rate. Implantation rate was higher in patient group having greater than 15% negatively charged sperm in their ejaculate (63/103 = 61.17%; n = 51) compared with patient group less than 15% negatively charged sperm (3/38 = 7.89%; n = 19) in their ejaculate. Couples achieving clinical pregnancy (n = 41) had a higher percentage of negatively charged sperm population in their ejaculate (56.63 ± 4.91 vs. 26.34 ± 6.31, p < 0.001) and lower percentage of positively charged sperm population (41.61 ± 4.87 vs. 69.66 ± 6.58, p = 0.001), than couples who did not achieve clinical pregnancy.
Conclusions: There is a statistically significant association between sperm's charge and clinical outcomes. Hypothetically, selection of negatively charged sperm to be used for assisted treatment has a potential to improve ART success.

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HUMAN BINDER OF SPERM PROTEIN HOMOLOG 1 (BSPH1) CAN PROMOTE SPERM CAPACITATION.

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(Presented By: Genevieve Plante)

Introduction: Binder of SPerm (BSP) proteins are a family of proteins expressed exclusively in the male reproductive tract of several mammalian species and are known to promote capacitation. Our recent studies have shown that in human, the Binder of SPerm Homolog 1 (BSPH1) is expressed specifically in epididymal tissues. Up until now, no studies had ever been done on the role of human BSPH1 in sperm functions.
Methods: To test this, a recombinant BSPH1 (rec−BSPH1) was produced, purified and refolded on−column using a decreasing urea gradient. First, the effect of rec−BSPH1 on sperm capacitation and tyrosine phosphorylation was evaluated. Results obtained showed that human rec−BSPH1 was able to promote sperm capacitation of ejaculated sperm and that this effect was dose−dependent. However, it had no effect on tyrosine phosphorylation. We then tested the effect of rec−BSPH1 on sperm motility using a Sperm Class Analyzer system. The protein was found to have no effect on any parameters of sperm motility tested (total motility, progressive motility or hyperactivation). Binding pattern of BSPH1 on ejaculated sperm was also tested by immunofluorescence microscopy.
Results: It was found to be localized on the equatorial segment, post−acrosomal segment and neck of the sperm.
Conclusion: These results show that the human epididymal BSPH1 shares functional characteristics with BSP proteins secreted by seminal vesicles of ungulates. (Supported by NSERC, CIHR and FESP of university of Montréal)

ABSTRACTS

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QUANTITATIVE PHOSPHOPROTEOMIC ANALYSIS OF SPERM CAPACITATION REVEALS A KEY ROLE OF IGF1R TYROSINE KINASE IN HUMAN

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(Presented By: Jing Wang, PhD Candidate)

Introduction and Objectives: Spermatozoa must reside in the female genital tract for a specific period of time acquire the ability of fertilizing eggs, and named 'Capacitation'. Several biochemical changes occur at specific time during sperm capacitation, such as cholesterol efflux, membrane ion infiltrative increases, and enhancement of tyrosine phosphorylation. Therein, one of the most important change is the enhancement of tyrosine phosphorylation. However, what the role of protein tyrosine phosphorylation in sperm capacitation is not clear. Aim is to discover new phosphorylation modification proteins and the key tyrosine phosphorylated kinases during sperm capacitation.
Methods: Here we empolyed labelfree quantitative phosphoproteomics to investigate the overall phosphorylation events during sperm capacitation. Totally, 3350 phosphorylated sites corresponding to 1017 phosphorylated proteins were identified (FDR<1%) using IMAC−TiO2 phosphopeptide continuous enrichment methods by LC−MS/MS.
Results: In capacitated spermatozoa, 86 phosphorylation proteins and 16 tyrosine phosphorylation proteins were up−regulated (median normalized ratio >2). The NetworKIN algorithm predicted the tyrosine phosphorylation kinases IGF1R and INSR involved in sperm capacitation. These results suggested that IGF1R and INSR may be important tyrosine phosphorylation kinases during sperm capacitation. Analysis of spermatozoa hyperactivation associated motility by CASA showed that GSK1904529A (inhibits IGF1R and IR) treatment either in containing IGF1 factor sperm or in containing insulin factor sperm caused a significant reduction of the motility parameter in a time−dependent manner. Simultaneously, IGF1 factor enhanced spermatozoa hyperactivation associated motility, but insulin factor didn't. Moreover, NVP−ADW742 (inhibits IGF1R specifically) treatment merely caused a significant reduction of spermatozoa hyperactivation associated motility parameter in containing IGF1 factor sperm. These results suggested IGF1R tyrosine kinases might be play a critical role during sperm capacitation. Western Blotting further confirmed these results.
Conclusion: IGF1R mediated tyrosine phosphorylation regulation pathways has played a key role and affected sperm hyperactivation associated motility during human sperm capacitation. Futhermore, it provide a candidate molecular target for clinical diagnosis and treatment of male contraception and male infertility.

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INSIGHTS INTO THE LYSINE ACETYLATION OF PROTEINS IN CAPACITATED HUMAN SPERM

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(Presented By: Xuejiang Guo, PhD)

Introduction: Protein lysine acetylation is a dynamic and revisable post−modification that is known to play diverse functions in eukaryotes. Nevertheless, the composition and function of non−histone lysine acetylation in gametes remain unknown.
Methods: In the present study, we found complex lysine acetylated proteins in human sperm. In human, only capacitated sperm have the capacity to fertilize an egg. After immunopurification enrichment of acetylpeptides with anti−acetyllysine antibody and high−throughout liquid chromatography−tandem mass spectrometry (LC−MS/MS) identification, we characterized 1206 lysine acetylated sites, corresponding to 576 lysine acetylated proteins in human capacitated sperm.
Results: Subcellular localization analysis showed that they mainly localize on mitochondrion (153 genes), microtubule (33 genes), flagellum (21 genes), nucleoplasm (25 genes), nucleosome (9 genes), cytosol (13 genes) and plasma membrane (8 genes). Most subunits of protein complexes such as respiratory chain complex I, proton−transporting ATP synthase complex and proteasome complex are acetylated. These identified acetylated proteins are associated with sperm functions, including motility, capacitation, acrosome reaction and sperm−egg interaction. Indirect immunofluorescence analysis of capacitated human sperm and mouse sperm revealed similar distribution of positive signals, with the strongest signals in the midpiece and principle piece. In vitro fertilization inhibition assay by anti−acetyllysine antibody showed essential functions of lysine acetylation in mouse sperm fertilization. And HDAC inhibitors, TSA and NAM, can significantly suppress sperm motility.
Conclusion: Lysine acetylation is expected to be an important regulator in sperm functions. And our characterization of lysine acetylproteome can be a rich resource for the studies of male fertility.

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SPERM MOTILITY LOSS AND ACTIVATION OF THE CAMP−PKA PATHWAY CAUSED BY THE STAT3 INHIBITORY COMPOUND V RESULT FROM EXCESSIVE REACTIVE OXYGEN SPECIES PRODUCTION.

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(Presented By: Catherine Lachance, PhD)

ABSTRACTS

Introduction: We previously showed that the Stat3 inhibitory compound (Stattic V) alters human sperm motility and mitochondrial activity. Higher levels of reactive oxygen species (ROS) were measured when sperm were incubated with the Stattic V, in agreement with the well−known increased production of ROS caused by mitochondrial dysfunction. Moreover, we recently observed that the negative effect of Stattic V on sperm motility was more pronounced when activators of the PKA pathway are present in the incubation medium. As the stimulation of the PKA pathway is also known to elevated ROS production in sperm, we hypothesized that the effect of Stattic V on sperm motility was caused, at least in part, by the elevated ROS production.
Methods: To address the role of elevated intracellular ROS on the different sperm functions affected by the Stattic V, a membrane permeable antioxidant, N−acetyl−L−cysteine (NAC), was added to the incubation medium. Following sperm incubation in different conditions, motility was evaluated visually, sperm acrosomal integrity was determined by FITC conjugated Pisum sativum agglutinin (PSA−FITC) staining and tyrosine phosphorylation of total proteins as well as serine/threonine phosphorylation of PKA substrates were evaluated by western blot.
Results: The effects of Stattic V on motility and the percentage of A23187−induced acrosome reaction were neutralized by the presence of NAC in the incubation medium. The phosphotyrosine content and the phosphorylation level of PKA substrates were also similar to those observed in the control condition when NAC was present with Stattic V in the incubation medium. We also observed that after one hour of pre−incubation with the Stattic V, the addition of NAC was not sufficient to prevent the gradual motility loss. Similarly, the phosphorylation level of PKA substrates depended on the length of exposition to Stattic V before the addition of the antioxidant.
Conclusion: Those results indicate that the effects of Stattic V on different sperm functions result directly or indirectly from excessive ROS production and that the motility loss and PKA activation caused by Stattic V are irreversible. Those results also suggest that the motility loss caused by Stattic V is PKA−independent and that the more pronounced effects of Stattic V on sperm motility observed when sperm were treated with PKA activators could results from a positive amplification loop of ROS production.

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ROBUST AUTOMATIC SPERM TRACKING

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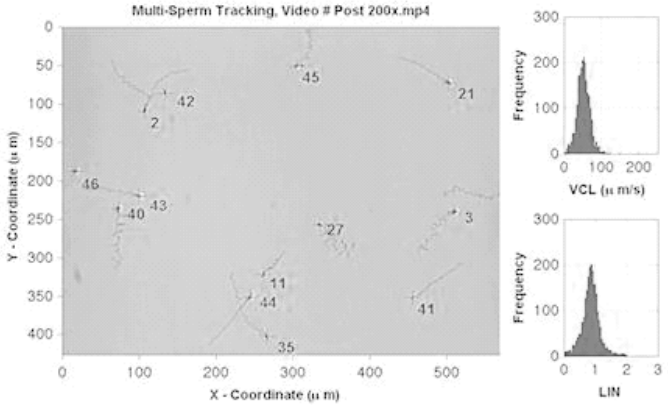
(Presented By: Leonardo Urbano, MSEE)

Objective: Our objective is to develop a fully−automated, robust, multi−sperm tracking algorithm capable of measuring sperm motility parameters accurately with minimal operator intervention. This effort is informed by progress in signal processing and target tracking technologies over the last three decades. A vast majority of sperm motility analysis is performed by technicians using subjective visual measurement−taking. Sometimes computer−assisted semen analysis (CASA) technology is used. However, most CASA systems are prohibitively expensive and require significant user intervention to track sperm whose paths have collided or are in close proximity. Target tracking algorithms developed originally for radar applications and video processing have addressed similar problems in other domains successfully and their methodologies can be used for sperm tracking and analysis.
Methods: Videos of washed sperm samples were recorded and digitized at 100x, 200x, and 400x magnification at 30 frames per second. A custom−made MATLAB algorithm was developed to automatically detect sperm in recorded video frames and perform multi−sperm tracking. A joint probabilistic data association (JPDA) filter − representing a mature technology employed in air traffic control systems − was used to reconcile sperm track−measurement association conflicts. This approach enabled accurate tracking of dozens of sperm simultaneously through collisions. In addition, tracks are automatically initiated and deleted as sperm enter and exit the video frame.
Results: Our algorithm is capable of tracking simultaneously every sperm in every video frame studied without any human intervention. Numbered sperm tracks were overlaid on the original video frames accompanied by an animated histogram of the curvilinear velocity (VCL) and path linearity (LIN) calculated for every sperm tracked. Our animated VCL and LIN histograms are useful for differentiating between samples of sperm based on motility.
Conclusions: The JPDA algorithm was effective at tracking simultaneously dozens of sperm through collisions while calculating VCL and LIN. To our knowledge, these results represent the first time JPDA has been applied to sperm tracking.



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JUSTICIA GANDARUSSA BURM.F. AS HYALURONIDASE HUMAN SPERMATOZOA INHIBITOR ACTIVITY

Bambang Prajogo
(Presented By: Bambang Prajogo)

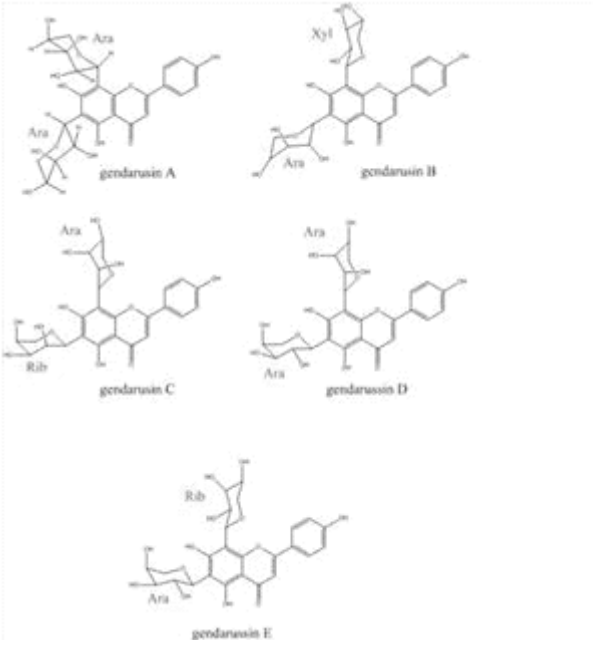
Introduction and Objective: Flavonoid glycoside is known as a hyaluronidase inhibitor which is an enzyme that has a role in human fertilization process. This enzyme present on the spermatozoa acrosomes digest hyaluronic acid substrate on the layer of the ovum. Justicia gendarusa Burm.f. leaf contains 12 components of flavonoid glycosides with the same molecular weight (MW 535). Gendarusin A is the major compound on it. In the preliminary research, isolate and extract showed the reversible competitive inhibitor activity in vitro. In the same activity also showed the decreasing of spermatozoa hyaluronidase on mice and human. Objective is to determine the decreasing of hyaluronidase human sperm activity which is inhibits the fertilization process.

Method: Research about anti−fertility which use capsule of 70% ethanol extract of J. gandarusa has been done. The dose of the capsule for 18 subjects was 450 mg/70 kg BW once a day for 30 days. The measurement used microplate with 96 wells to determine the catalytic and specific activity of the enzyme by spectrophotometer at Î» 595 nm. The subjects administered the capsules for 30 days. Assay of hyaluronidase activity was performed at the day 0, 15, 30 and 60.

Result: The study showed that the catalytic activity of hyaluronidase before taking the capsule was 1.5506.10−6 unit/million of spermatozoa. After taking the capsule for 15 days, the hyaluronidase activity was 1.4600.10−6 unit/million of spermatozoa and 1.48889.10−6 unit/million of spermatozoa at day 30. At day 60, i.e. 30 days after stopping the treatment, the activity was 2.7994.10−6 unit/million of spermatozoa. While the specific activity of hyaluronidase before taking capsule was 9.6672.10−8 unit/mg, after taking the capsule, on day 15th was 9.4911.10−8 unit/mg and on day 30th was 8.9350.10−8 unit/mg. At day 60, i.e. 30 days after stopping the treatment, the activity was 9.8056.10−8 unit/mg.

Conclusion: In conclusion, activity of hyaluronidase enzyme decreased after consuming the capsule. It was known that after stopping the capsule administration for 30 days, hyaluronidase enzyme was back to normal.

Keywords: Justicia gendarussa Burm.f., hyaluronidase, human spermatozoa, anti−fertility, gendarusin



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REGULATION OF ACROSOME REACTION BY LIPRIN A3, LAR AND ITS LIGANDS IN MOUSE SPERM

Chetanchandra Joshi, Msc, Shagufta Khan, PhD and Vrinda Khole, PhD
National Institute for Research in Reproductive Health
(Presented By: Chetanchandra Joshi, Msc)

Introduction and Objectives: Zona pellucida (ZP) based induction of acrosome reaction (AR) is a popular and well accepted hypothesis. However, this hypothesis is being challenged in recent years and it has been proposed that the cumulus cells might be the site of AR. In the present study we demonstrate the Liprin α3 interaction with RIM and LAR and show the importance of interaction of Liprin α3 and LAR in acrosome reaction. The present study was designed to understand the role of Liprin α3 and its interacting proteins LAR, RIM in regulation of AR.

Methods: 1. Western blot analysis & Indirect Immunofluorescence (IIF) of LAR was carried out on tissue and sperm. 2. Co−localization of LAR, Rab Interacting Molecule (RIM) with Liprin α3 was carried out. The extent of overlap was calculated. 3. Mouse cumulus cells were analysed for the presence of Syndecan−1 with Anti Syndecan−1 antibody 4. To check the effect of LAR ligands i.e. Syndecans and nidogens and LAR wedge peptide capacitated sperm were spiked with different concentrations of recombinant ligands, wedge peptide & anti Liprin α3. Acrosome exocytosis index was then calculated and effect was considered significant at $p<0.05$

Results: It is observed that the presence of anti−Liprin α3 antibody inhibits the process of acrosome reaction. Co−localization experiments demonstrate the co−existence LAR (Leucocyte Antigen Related), Rab Interacting Molecule (RIM) and Liprin α3 on sperm acrosome thereby completing the identification of all the members of RIM/MUNC/Rab3A/liprinα complex required for membrane fusion. Our study demonstrates an increase in AR in presence of LAR ligands such as Syndecans, Nidogens and LAR wedge domain peptide on acrosome reaction. Based on these data we speculate that in presence of ligands or wedge peptide, LAR undergoes dimerization leading an increase in AR.

Conclusions: Overall this study demonstrates that sperm acrosome reaction is driven by common set of proteins like Liprin α3, LAR, RIM shown to be responsible for membrane fusion at synapse. We could also demonstrate that the ligands and wedge peptide can induce LAR dimerization and could be one of the mechanisms of stimulating acrosomal exocytosis. The observations support the hypothesis that cumulus could be another site of acrosome reaction.

Acknowledgement: Financial assistance provided to CJ by Department of Science and Technology, New Delhi as DST−JRF, and Indian Council of Medical Research for ICMR−SRF and NIRRH, Mumbai is gratefully acknowledged.

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ASSESSMENT OF SPERM DNA FRAGMENTATION AFTER MICROSCOPIC SUBINGUINAL VARICOCELECTOMY
INTRODUCTION:

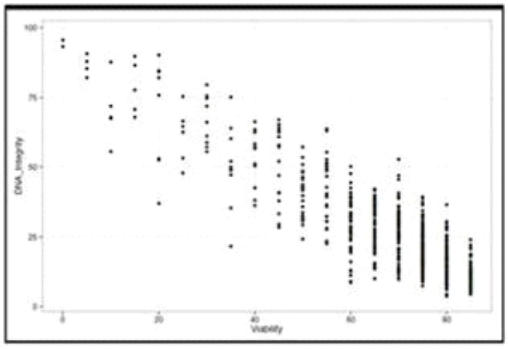
Brooke Harnisch, MD and Jay Sandlow, MD
Medical College of Wisconsin
(Presented By: Brooke Harnisch, MD)

Objective: To evaluate DNA fragmentation in male infertility patients before and after microscopic subinguinal varicocelectomy.

Methods: An institutional review board (IRB) approved retrospective study was conducted on infertile men with palpable varicoceles who underwent microscopic subinguinal varicocelectomy between September 2012 and June 2013. Exclusion criteria included: adolescents and patients undergoing surgery for pain. Demographic, clinical and laboratory data was collected. DNA fragmentation was determined by Halosperm® diagnostic kit.

Results: A total of eight patients were identified who had complete pre and post op information. Mean total sperm count and sperm concentration significantly improved after varicocelectomy from 13.7 x106 to 29.5x106 and 4.4x106/ml to 8.6x106/ml ($p<0.05$). Total progressively motile sperm per ejaculate trended to significance from 2.7 x106 to 7.9x106 ($p=0.07$). Overall, there was no significant change in sperm DNA fragmentation after surgery. On subgroup analysis, patients with a DNA fragmentation <20% and a DNA fragmentation >20% had no significant improvement post−operatively. However, despite having similar pre−operative mean sperm count and concentration, patients with DNA fragmentation <20% had a significantly higher post−operative sperm count than patients with a DNA fragmentation >20% ($p=0.03$).
Conclusions: Varicocelectomy significantly improves semen parameters but does not decrease DNA fragmentation levels. Randomized controlled trials are needed before impaired sperm DNA integrity may be considered as an alternative indication for varicocele repair.

Figure 1: Sperm DNA fragmentation versus sperm viability



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THE RELATIONSHIP BETWEEN SPERM VIABILITY AND DNA FRAGMENTATION RATES

Mary Samplaski, MD[1], Apostolos Dimitromanolakis, MSc[2], Brendan Mullen, MD[2], Kirk Lo, MD[2], Ethan Grober, MD[2] and Keith Jarvi, MD[2]
[1]Mount Sinai Hospital, University of Toronto, Toronto, Ontario; [2]Mount Sinai Hospital, University of Toronto
(Presented By: Mary Samplaski, MD)

Introduction: We sought to determine the relationship between sperm viability and DNA fragmentation index (DFI). Specifically we evaluated the relationship between viability and DFI > 30%, since a DFI > 30% has been associated with the need for intracytoplasmic sperm injection.
Methods: Men having semen analyses with both vitality and DFI testing were identified. Viability was measured by the eosin−nigrosin assay. DNA fragmentation was measured using a sperm chromatin structure assay with flow cytometry. The relationship between DFI and viability was assessed by univariate analysis.
Results: A strong inverse relationship ($p<0.001$) was seen between viability and DNA fragmentation rates, with Pearson correlation coefficient $r=-0.87$ (Figure 1). A total of 3050 men had both DFI and viability assays. If viability was very high (≥ 80%, n=1104) then DFI was consistently ≤ 30% (100% sensitivity to predict DFI ≤ 30%). If viability was ≥ 75% (n=1736), then the DFI was ≤ 30% for 95% of the patients. For samples with very low viability (viability ≤ 35%, n=91) then DFI was always ≥ 30%. If viability was ≤ 50% then DFI was ≥ 30% for 95% of the samples (n=310).
Conclusions: Sperm viability correlates strongly with DNA fragmentation rates. In men with sperm viability ≤ 50%, 95% of the time the DFI is ≥ 30%; Conversely, if sperm viability ≥ 75%, 95% of the time the DFI is ≤ 30%.

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CHARACTERIZATION OF MEMBRANE OCCUPATION AND RECOGNITION NEXUS REPEAT CONTAINING 3, A MEIG1 BINDING PARTNER, IN MOUSE MALE GERM CELLS

Ling Zhang, Hongfei Li, MD, Yuqin Shi, PhD, Maria Teves, PhD, Zhiqiong Wang, MD, Gaofeng Jiang, PhD, Shizhen Song, PhD and Zhibing Zhang, PhD
(Presented By: Ling Zhang)

Introduction: Mammalian spermatogenesis is a well−organized process of cell development and differentiation; the morphogenesis of spermatozoa is the final step of spermatogenesis. During this process, haploid round spermatids differentiate into spermatozoa, with dramatic morphological changes. Meiosis, expressed gene 1 (MEIG1), plays an essential role in the regulation of this step.
Methods: To explore potential mechanisms of MEIG1 in the regulation of spermiogenesis, a yeast two−hybrid screen was conducted and several potential binding partners were identified; one of them was membrane occupation and recognition nexus repeat containing 3 (MORN3). The interaction between MORN3 and MEIG1 was confirmed by co−immunoprecipitation in cultured mammalian cells over−expressing the two proteins. Morn3 mRNA is only abundant in mouse testis. In the testis, Morn3 mRNA is highly expressed in the spermiogenesis stage. Specific anti−MORN3 polyclonal antibody was generated against N−terminus of the full length MORN3 protein, and MORN3 expression and localization was examined in vitro and in vivo. In transfected CHO cells, the antibody specifically crossed−reacted the full length MORN3 protein, and immunofluorescence staining revealed that MORN3 was localized throughout the cytoplasm.
Results: Among multiple mouse tissues, an about 25 kDa protein, but not the full length 28 kDa MORN3 protein was identified only in the testis. The protein was highly expressed after day 20 of birth. Immunofluorescence staining on mixed germ cells isolated from adult wild−type mice demonstrated that MORN3 was not present in spermacytes, but expressed in the acrosome in germ cells throughout spermiogenesis. The protein was also present in the manchette of elongating spermatids. The total MORN3 expression and acrosome localization were not changed in the Meig1−deficient mice. However, its expression in manchette was dramatically reduced in the mutant mice.

Conclusion: Our studies suggest that MORN3 might be another regulator for spermatogenesis, probably together with MEIG1.

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A MEIG1/PACRG COMPLEX IN THE MANCHETTE IS ESSENTIAL FOR THE TRANSPORT OF STRUCTURAL PROTEINS REQUIRED FOR CONSTRUCTION OF THE SPERM FLAGELLA

Maria Teves, PhD, David Nagarkatti−Gude, Kellie Archer, Waixin Tang, Darrell Peterson, Jerome Strauss, Zhibing Zhang
(Presented By: Zhibing Zhang)

Introduction: One of the hallmarks of spermiogenesis is the formation of flagella, which enables sperm to reach eggs for fertilization. The molecular mechanism of flagellogenesis is poorly understood. Meiosis−expressed gene 1 product (MEIG1) is a key regulator of spermiogenesis. Meig1−deficient male mice are sterile as a result of impaired spermiogenesis. Dynamic analysis of testicular histology revealed that the testes from Meig1−deficient mice have abnormal morphological after 28 days of birth, the time when germ cells enter the stage of elongation/condensation. Except Meig1, DNA microarray assays did not identify other genes whose expression in the testes was significantly changed at both 22 and 28 days after birth in the mutant mice. We previously discovered that Parkin co−regulated gene (PACRG) was the major binding partner of MEIG1.
Methods: Using PACRG as bait, MEIG1 was also identified to be its major binding partner. Male mice deficient in PACRG display a similar reproductive phenotype to that of Meig1−deficient mice. In spermatocytes of wild type mice, MEIG1 is expressed in the whole cell bodies, but it migrates to the manchette in the elongating spermatids. PACRG protein appears during the transition of round spermatids into elongating spermatids, which is much later than the appearance of Pacrg transcript, suggestive of translational or posttranslational control of expression of this gene.
Results: In the elongating spermatids of wild−type mice, PACRG co−localizes with α−tubulin, a marker for manchette, this localization was not changed in the remaining elongating spermatids of Meig1−deficient mice. However, MEIG1 is no longer localized in the manchette in the remaining elongating spermatids of Pacrg−deficient mice, indicating that PACRG recruits MEIG1 to the manchette. PACRG is not stable in either bacteria or mammalian cells, but can be stabilized by MEIG1. Besides PACRG, MEIG1 also associates with SPAG16L, a sperm axonemal central apparatus protein. SPAG16L is present in the spermatocyte cytoplasm of wild−type mice, and in the manchette of elongating spermatids, but in the Meig1−deficient mice, SPAG16L is no longer localized in the manchette. However, MEIG1 is still present in the manchette of Spag16L−deficient mice, suggesting that SPAG16L is a downstream partner of MEIG1.
Conclusion: Our studies demonstrate that MEIG1 and PACRG form a complex in the manchette, and that this complex is essential to transport sperm flagellar proteins, like SPAG16L, to build the sperm flagella.

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COMBINED ADMINISTRATION OF CURCUMIN AND GALLIC ACID INHIBITS GALLIC ACID−INDUCED SUPPRESSION OF STEROIDOGENESIS, SPERM OUTPUT, ANTIOXIDANT DEFENSES AND INFLAMMATORY RESPONSIVE GENES

Sunny Abarikwu, PhD, Mojisola Durojaiye, BSc, Adenike Alabi, BSc, Oghenetega Akiri, BSc
Redeemer's University, Nigeria
(Presented By: Sunny Abarikwu, PhD)

Introduction: In this study, we investigated the effects of administration of gallic acid (Gal) with or without curcumin (Cur) on the sperm output, steroid level and antioxidant defenses in rat testis in vivo and the expression of inflammatory responsive genes in vitro.
Methods: Male Wistar rats were divided randomly into four groups and given oral Gal (100 mg/kg/day) and Cur (100 mg/kg/day) alone or in combination for four weeks. The sperm quality was impaired following Gal treatment, while Cur prevented this and also improved the sperm count as well as the efficiency of sperm production (DSP/gm testis). The inhibitory effects of Gal on plasma testosterone level, glutathione levels, activities of glutathione peroxidase, catalase, superoxide dismutase and steroidogenic enzymes, 3β−hydroxysteroid dehydrogenase (3β−HSD) and 17β−HSD in the rat testis was blocked by Cur.
Results: Interestingly, the level of testosterone and the activities of the steroidogenic enzymes were significantly increased after treatment with Cur alone. Malondialdehyde concentration was unchanged following Gal treatment, while a significant decrease in malondialdehyde level was observed following treatment with Cur alone or in combination with Gal. We further analyzed the effects of Cur and Gal (25−100 μM) on the 93RS2 Sertoli cell−lines and observed that Cur blocked the Gal−induced suppression of inflammatory mediators such as TNF−α and IL−6, while Gal blocked the suppressive effect of Cur on IL−1α expression. Furthermore, the stimulatory or inhibitory effects of Gal on the expressions Tgf−β1 and CD−14 was concentration−dependent and could be blocked by Cur. When cultures of primary Sertoli cells were exposed to both Cur and Gal for 24 h, p−JNK/SAPK expression remain stable, whereas Gal−induced p−p65 (NF−ÎºB) expression and IÎºBα degradation was seen to be blocked by Cur but not Gal−induced expression of pERK1/2.
Conclusion: Overall, Cur has stimulatory reproductive effects and could protect the testis from the toxic effects of Gal by mechanisms that could not be explained by its effects on the expressions of inflammatory cytokines but by its anti−oxidant properties.

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THE TRANSCRIPTION FACTOR MEF2 IS A NOVEL REGULATOR OF GSTA1 EXPRESSION IN MA−10 LEYDIG CELLS

Mickael Di−Luoffo, MSc, Catherine Brousseau, MSc, Francis Bergeron, MSc and Jacques J. Tremblay, PhD
CRCHUQ−Universite Laval
(Presented By: Mickael Di−Luoffo, MSc)

Introduction: Testosterone is essential for spermatogenesis and for the development of primary and secondary male sexual characteristics. Steroidogenesis, however, produces a significant amount of reactive oxygen species (ROS), which in turn disrupt testosterone production.

Methods: Our lab has identified members of the Myocyte Enhancer Factor 2 (MEF2) family of transcription factors in the mouse testis. MEF2 factors are important regulators of organogenesis and cell differentiation in various tissues. In the testis, MEF2 is present in Sertoli and Leydig cells throughout fetal and adult life suggesting a role for this factor in somatic cell differentiation and function. Supporting this, we found that MEF2 regulates the expression of genes involved in steroidogenesis. Furthermore, analysis of the transcriptome of MEF2−deficient (siRNA knockdown) MA−10 Leydig cells revealed a significant decrease in the expression of Gsta family members (glutathione−S−transferase) that encode ROS inactivating enzymes. The aim of the present study was to determine the role of MEF2 in Gsta1 expression in Leydig cells.
Results: By qPCR, we confirmed that Gsta1 mRNA level was decreased by 74% in MEF2−deficient MA−10 Leydig cells. Conversely, overexpression of MEF2 in these cells lead to a 1.5 fold increase in endogenous Gsta1 mRNA levels. In silico analyses of the Gsta1 promoter revealed the presence of a consensus MEF2 binding site (YTAWWWWTAR) at −506 bp. MEF2 recruitment to the proximal Gsta1 promoter was confirmed by ChIP whereas no significant recruitment was observed on a distal Gsta1 promoter region lacking MEF2 element or when an IgG was used. Next a 2 kb fragment of the mouse Gsta1 promoter was isolated and fused to luciferase for functional studies. Mutation of the MEF2 element at −506 bp led to a 68% decrease in Gsta1 promoter activity. In addition, transfection of MEF2 in MA−10 cells led to a 2.2 fold activation of the Gsta1 promoter, which was lost when the MEF2 element was deleted or mutated. These data indicate that the MEF2 element at −506 bp is essential for MEF2 responsiveness. Since MEF2 can be activated by CAMKI (which is present in Leydig cells), MEF2 and CAMKI were co−transfected in MA−10 cells and this resulted in a 5.7 fold activation of the Gsta1 promoter.
Conclusion: In conclusion, our results identify a novel role for MEF2 in the regulation of genes involved in Leydig cell detoxification, a process essential for the maintenance of testosterone production. Supported by CIHR.

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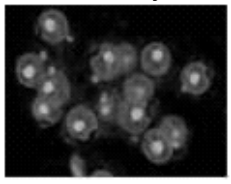
DETECTION OF STRONGLY REPRESSED AND HIGHLY ACTIVE MRNAS IN THE CHROMATOID BODY OF ROUND SPERMATIDS WITH A SIMPLE AND SENSITIVE FLUORESCENT IN SITU HYBRIDIZATION TECHNIQUE.

Danielle Cullinane, Graduate Student and Ken Kleene, PhD
Umass Boston
(Presented By: Danielle Cullinane, Graduate Student)

Introduction and Objectives: Many mRNAs are stored as translationally inactive free−mRNPs in round spermatids and actively translated in elongating and elongated spermatids. A popular idea is that free−mRNPs are repressed by storage in the chromatoid body, a cytoplasmic mRNP−granule in round spermatids that is devoid of ribosomes, and is thought to coordinate mRNA translation and degradation. A notable gap in this model is the paucity of evidence that mRNAs are even present in the chromatoid body. The objectives of this study are to develop reliable fluorescent in situ hybridization (FISH) techniques to detect mRNAs in the chromatoid body and to compare the localization of mRNAs that are strongly repressed and actively translated in round spermatids.
Methods: Dried down preparations of stage II−VI seminiferous tubules were analyzed with FISH using tiled fluorescently labeled antisense oligo probes for four mRNAs: the sperm mitochondria−associated cysteine−rich protein (Smcp) mRNA and a Smcp−Gfp transgenic mRNA, both of which are both strongly repressed in round spermatids, and the lactate dehydrogenase C (Ldhc) mRNA and another Smcp−Gfp transgenic mRNA, both of which are highly active in round spermatids. FISH was detected with conventional and confocal fluorescence microscopy, and correlated with immunological markers for the chromatoid body (DDX4/MVH) and free−mRNPs (Y−box protein 2, YBX2/MSY2).
Results: All four mRNAs exhibit intense FISH in a small irregular, perinuclear spot in round spermatids which overlaps DDX4. In contrast, YBX2 is present throughout the cytosol with a small amount in the chromatoid body. Interestingly, DDX4, YBX2 and mRNAs are differentially localized within the chromatoid body.
Conclusions: We suggest a counterintuitive interpretation of these findings. The strong FISH signal of all four mRNAs in the chromatoid body represents a high concentration of a small number of mRNA molecules in a very small volume, while the weak signal in the cytosol represents a low concentration of a larger number of mRNA molecules in free−mRNPs and polysomes in a much larger volume. Conceivably, mRNPs are transiently stored and remodeled in multiple compartments in the chromatoid body.



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A−SINGLE SPERMATOGONIA HETEROGENEITY AND CELL CYCLE SYNCHRONIZE WITH A SPECIFIC RAT SEMINIFEROUS EPITHELIAL STAGE

Shadaan N. Abid, PhD, Timothy E. Richardson, MD, PhD, Heather M. Powell, MS, Priscilla Jaichander, PhD, Jaideep Chaudhary, BS, Karen M. Chapman, BS and F. Kent Hamra, PhD
UT Southwestern Medical Center in Dallas
(Presented By: F. Kent Hamra, PhD)

Introduction: In mammalian testes, type A−single spermatogonia function as stem cells that sustain sperm production for fertilizing eggs. Currently, it is not understood how cellular niches regulate the developmental fate of A−single spermatogonia.
Method: Here, anatomical maps and immunolabeling studies in rat testes define a novel population of ERBB3+ germ cells as ~5% of total SNAP91+ A−single spermatogonia along a spermatogenic wave.
Results: As a function of time, ERBB3+ A−single spermatogonia are transiently detected during a 1−2 day period each 12.9 day sperm cycle, representing 35−40% of SNAP91+ A−single spermatogonia in stage VIII seminiferous tubules. ERBB3+ spermatogonia also synchronize their cell cycle during this epithelial stage where they form physical associations with preleptotene spermatocytes transiting the blood−testis−barrier, and Sertoli cells undergoing sperm release.
Conclusion: Thus, induction of stem cell heterogeneity within this specific, short−lived and re−occurring microenvironment highlights novel theories on how cellular niches could integrate with testicular physiology to orchestrate sperm development in mammals.

ABSTRACTS

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LINKING SPERMATID RNA BINDING PROTEIN DIVERSITY TO REPRODUCTIVE SUCCESS

Karen M. Chapman, BS, Jaideep Chaudhary, BS, Timothy E. Richardson, MD, PhD and F. Kent Hamra, PhD
UT Southwestern Medical Center in Dallas
(Presented By: F. Kent Hamra, PhD)

Introduction: Spermiogenesis is a postmeiotic process that drives development of round spermatids into fully elongated spermatozoa. Spermatid elongation is largely controlled post−transcriptionally after global silencing of mRNA synthesis from the haploid genome.
Methods: Here, rats that differentially express EGFP from a lentiviral transgene during early and late steps of spermiogenesis were used to flow sort fractions of round and elongating spermatids. Mass−spectral analysis of 2D gel protein spots enriched >3−fold in each fraction revealed a heterogeneous RNA binding proteome (hnRNPA2/b1, hnRNPA3, hnRPDL, hnRNPK, hnRNPL, hnRNPM, PABPC1, PABPC4, PCBP1, PCBP3, PTBP2, PSIP1, RGSL1, RUVBL2, SARNP2, TDRD6, TDRD7) abundantly expressed in round spermatids prior to their elongation.
Results: Notably, each protein within this ontology cluster regulates alternative splicing, subcellular transport, degradation and/or translational repression of mRNAs. In contrast, elongating spermatid fractions were enriched with glycolytic enzymes, redox enzymes and protein synthesis factors. Retrogene−encoded proteins were over−represented among the most abundant elongating spermatid factors identified.
Methods: Consistent with these biochemical activities, plus corresponding histological profiles, the identified RNA processing factors are predicted to collectively drive post−transcriptional expression of an alternative exome that fuels finishing steps of sperm maturation and fitness.

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EFFECTS OF ALLII TUBEROSI SEMEN ON THE CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) EXPRESSION DURING SPERMATOGENESIS

Jin hyoung Cho, MS, Sung Won Jee, PhD, Do Rim Kim, PhD, Ha Young Kim, MS, Eun Bit Ko, MS, Ho Jin Lee, MS, Mun Seog Chang, PhD, Seong Kyu Park, PhD
Department of Prescriptionology, College of Korean Medicine, Kyung Hee University
(Presented By: Seong Kyu Park, PhD)

Introduction: The cyclic AMP response element modulator (CREM) is a transcription factor highly expressed in the post−meiotic germ cells of the testis. CREM is a key factor in spermatogenesis and a causal factor of round spermatid maturation arrest in idiopathically infertile men.
Methods: In order to investigate the effects of Allii tuberosi Semen (AS) on CREM expression, real−time PCR and Western blotting assays were performed in this study. C57BL/c mice were divided into four groups, the normal group and AS treated groups (100, 500, 1000 mg/kg of AS) for five weeks.
Results: In our results, sperm count and motility were increased in 100, 1000 mg/kg of AS treated group than that of normal group (178.56 ± 23.90, 225.42 ± 51.00 ×106 vs. 166.82 ± 37.22 and 64.75 ± 3.64, 68.87 ± 4.02 vs. 53.22 ± 1.74%, respectively.).
Conclusion: CERM expression level was significantly increased in 100, 1000 mg/kg of AS treated group than that of normal group. In conclusion, our results suggest that AS can promote spermatogenesis and increases sperm motility through the induction of CREM transcription factor.

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PEROXISOME PROLIFERATOR−ACTIVATED RECEPTOR−B/D (PPARB/D) REGULATES SPERMATOGENESIS BY ALTERING CELL−CYCLE REGULATORS IN MICE

Pei−Li Yao, LiPing Chen, Frank Gonzalez, Jeffrey Peters
(Presented By: Pei−Li Yao)

Introduction: Peroxisome proliferator−activated receptors (PPARs) are nuclear hormone receptors which control a variety of biological processes, including cell differentiation and embryo development. Although Pparβ/Î´−/− mice are fertile, they display a significantly smaller litter size compared to Pparβ/Î´+/+ mice.
Methods: Here, we showed that Pparβ/Î´−/− mice exhibit multi−nucleated giant germ cells, cell cycle arrest, germ cell depletion, vacuolization in Sertoli cells, and mixed−stages of spermatogenesis in the seminiferous tubule compared to Pparβ/Î´+/+ mice. This indicates that PPARβ/Î´ has a critical role in the functional spermatogenesis during testis development.
Results: The overall incidence of atrophic testes and testis degeneration in Pparβ/Î´−/− mice is significantly higher than that in Pparβ/Î´+/+ mice. At both peri−pubertal and adult ages, testicular CYCLIN D1 expression is limited in spermatogonia and is higher in Pparβ/Î´−/− mice than in Pparβ/Î´+/+ mice. Sertoli cells in Pparβ/Î´−/− mice express less p27 and the average number of Sertoli cells in seminiferous tubules of Pparβ/Î´−/− mice is higher than that in Pparβ/Î´+/+ mice. The expression of carcinoma in situ marker, placental alkaline phosphatase (PLAP), is stronger in Pparβ/Î´−/− mice testes than in Pparβ/Î´+/+ mice testes. The testicular cKIT expression is also higher in Pparβ/Î´−/− mice than in Pparβ/Î´+/+ mice.
Conclusion: Combined, these novel data suggest that PPARβ/Î´ regulates spermatogenesis by maintaining the homeostasis between the developing germ cells and the matured Sertoli cells in the seminiferous epithelium and may play a role in preventing the occurrence of carcinoma in situ.

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EFFECT OF IRRADIATION ON THE LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MOUSE TESTIS

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Ben−Gurion University of the Negev
(Presented By: Mahmoud Huleihel, PhD)

Introduction and Objectives: Vascular endothelial growth factor (VEGF) is a protein produced by a wide range of cells. It promotes vasculogenesis and angiogenesis. VEGF also causes proliferation of endothelial cells and increase the permeability of the wall of blood vessels. Although the function of VEGF in the testis is unknown, this factor is attributed with survival and development of testicular germ cells as well as determining fertility in mice. VEGF was detected in Sertoli, Leydig and some testicular germ cells. Irradiation affects dividing cells. In the testes of adults, the main affected cells are the developing germ cells. Irradiation was also shown to affect some functions of Sertoli and Leydig cells. However, the effect of irradiation on testicular VEGF was not yet examined. Objective is to evaluate the effect of irradiation on mouse testicular VEGF levels and cellular localization.
Methods: Mice (BALB/c; 8 weeks−old) were exposed (total body irradiation) once (at the beginning of the experiment) to different doses of irradiation [control group; (CT), 0.5, 2.5 and 10 Gy). After 1−10 weeks of irradiation, mice were sacrificed, and testes were weighted and collected to be evaluated: 1) Histologically by using hematoxylin−eosin staining; 2) For the levels of VEGF in the testicular tissue by ELISA; 3) For cellular localization by Immunohistochemical staining using specific anti mouse VEGF antibodies.
Results: Our results show that irradiation damages the normal structure of the seminiferous tubules and that the strongest effect of high irradiation doses on testicular weight and seminiferous tubules was detected 3−4 weeks post−irradiation, after that there was a recovery. Irradiation significantly increased the levels of VEGF in testicular homogenates. The effect of the different doses of irradiation (low and high) on VEGF levels was expressed in different time points post−irradiation. In addition, we showed that VEGF levels in testes of normal mice decreased with age increase. The main increase of VEGF was detected in interstitial cells and spermatocytes.
Conclusions: Our results support the suggestion that VEGF could be involved in the regulation of spermatogenesis, under normal and pathological conditions, through regulation Leydig cell activities and germ cell niches which may affect their growth, proliferation and/or differentiation.

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INHIBITION OF MTOR SIGNALING DECREASES STRA8 EXPRESSION IN ADULT MOUSE TESTIS

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(Presented By: Pinar Sahin, MSc)

Introduction: Mammalian target of rapamycin (mTOR) signaling serves as a regulator of growth and proliferation. Several studies have emphasized destructive impact of mTOR inhibitor, rapamycin, on male gonadal function in men. Recently, we showed that mTOR pathway components are localized in spermatogonia and preleptoten spermatocytes suggesting that mTOR pathway may have a role during proliferation and meiotic initiation of spermatogonia. Thus; we aimed to investigate the effect of mTOR inhibition to Stra8 expression utilizing seminiferous tubule culture system.
Methods: First, distribution of mTOR signaling molecules were evaluated in testes of adult mice by immunohistochemistry. Then, to evaluate the effect of mTOR inhibition on spermatogenic cells using seminiferous tubule culture experiments, 4 groups were established; control, 24 hour culture, rapamycin treated, and ethanol treated as vehicle. Up to five seminiferous tubule fragments were cultured in 30µl hanging drops and afterwards effects of rapamycin were examined using western blot analysis for p−p70S6K, PCNA, Stra8 and VASA. Cell viability assay and TUNEL was also performed in all groups.
Results: Firstly; our immunohistochemistry results showed that mTOR, p−mTOR, p−p70S6K, p−4EBP−1 proteins were localized in spermatogonial cells and preleptoten spermatocytes in adult mice testis. Secondly; seminiferous tubule culture experiments showed that cell viability was similar between the groups. Expression of p−p70S6K decreased significantly in rapamycin treated group indicating that mTOR signaling has been inhibited successfully. Furthermore, PCNA and Stra8 expressions decreased significantly in rapamycin treated group. No differences were observed for VASA expression between the groups. For all groups, the number of TUNEL positive cells was similar.
Conclusions: Our seminiferous tubule culture studies indicated that mTOR signaling may regulate spermatogonial stem cells by not only controlling their proliferative capacity but may also regulate their differentiation by controlling the expression of meiosis initiation molecule Stra8. Regulation of meiosis by this pathway is a novel finding and extensively under investigation in our laboratory utilizing in vitro and in vivo approaches. This study is supported by TUBITAK with the project numbers: 110S309 and 113S490, and Akdeniz University Scientific Research Projects with the project number 2010.02.0122.009.

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FUNCTIONAL CHARACTERIZATION OF ION CHANNELS IN SINGLE SPERMATOGONIA IN VITRO AND IN SITU.

David Fleck, MSc[1], Sophie Veitinger, PhD[2], Thomas Veitinger, PhD[1], Patricia Almeida Machado, BSc[1], Susanne Lipartowski[1], Corinna Engelhardt[1], Jennifer Spehr, PhD[1] and Marc Spehr, PhD[1]
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(Presented By: David Fleck, MSc)

Introduction: Spermatogenesis is a fundamental and highly complex biological process that ensures male fertility. Spermatogonia are the precursors of all male germ cell stages. Their differentiation assures the lifelong production of mature sperm. However, few physiological details are known about testicular cell communication during spermatogenesis. Since we and others have previously shown that Sertoli cells are able to communicate via ATP, we hypothesize a general role for purinergic signaling in the testis.
Methods: Using wildtype C57BL/6 mouse pups, we first developed a coculture of Sertoli cells and spermatogonia. Next, we investigated ATP−dependent signaling by whole−cell patch−clamp recordings from cultured spermatogonia. Pharmacological profiling and gene expression knockdown allowed identification of involved ion channels.

Results: Here, we report that cultured spermatogonia respond to extracellular ATP (1 − 100 μM). ATP−induced currents show fast activation and moderate desensitization. The current−voltage relationship reveals strong inward rectification. Current potentiation by ivermectin and inhibition by an acidic extracellular pH (6.3) and extracellular copper (100μM) indicate a functional role of P2X4 receptors. Accordingly, knockdown of P2X4R expression by RNA interference significantly reduced currents activated by ATP concentrations ≤ 300 μM. Interestingly, an increased ATP concentration (>300 μM) activated an additional current with different kinetics. A similar current could be activated by 300 μM 3'−O−(4−Benzoyl)benzoyl ATP (BzATP). Knockdown of P2X7R expression decreased the current activated by higher ATP concentrations (>300 μM). Combined with molecular evidence, our results indicate that at least two different of P2X receptor subunits (P2X7R and P2X4R) are functionally expressed in spermatogonia of young prepubescent mice. Downstream of P2X receptor activation, we found a slowly activating calcium−dependent potassium current functionally antagonizing the depolarizing P2XR−mediated current.
Conclusion: To confirm these results in situ, we established a new experimental approach. Using acute tissue slices of prepubescent mouse testis we electrophysiologically analyzed spermatogonia and found ATP−induced currents with similar characteristics. Together, these data represent a first important step towards a deeper understanding of cellular purinergic communication during spermatogenesis.

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FURTHER CONFIRMATION OF SEVERAL IMPORTANT TARGETS OF SUMOYLATION IN TESTICULAR CELLS

Yuxuan Xiao, PhD[1], Daniel Pollack, BsC[1], Avi Levy[1], Miriam Andrusier[1] and Margarita Vigodner, PhD[2]
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(Presented By: Margarita Vigodner, PhD)

Introduction: Sumoylation (a covalent modification by Small Ubiquitin−like Modifiers or SUMO proteins) has emerged as a critical regulatory event in cell function and has been implicated in various diseases; however, its role in reproduction is largely unknown.
Methods: In a previous study in our laboratory, using the STAPUT separation technique based on a gravity sedimentation followed by immunoprecipitation with SUMO antibody and mass spectrometry analysis, multiple SUMO targets were identified in meiotic spermatocytes and round spermatids. The identified targets of sumoylation included proteins involved in regulation of transcription, metabolism and stress response. Several specific targets with an important role in germ cells were chosen for further characterization.
Results: Co−Immunoprecipitation analysis confirmed sumoylation of CDC2 and CDC5l, the large subunit of RNA Polymerase II, Piwil2, MDC1 and several other proteins with an important role in regulating spermatogenesis.
Conclusion: Bioinformatic analysis revealed the presence of one or several consensus sequences for sumoylation in the majority of the studied targets.

Monday, April 7, 2014
11:00 a.m. - 12:30 p.m.

Poster Session II*
**Not CME Accredited*
Location: Venetian

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THE TRANSCRIPTION FACTOR SOX9 IS A NOVEL REGULATOR OF STEROIDOGENIC GENES EXPRESSION IN MA−10 LEYDIG CELLS

David Landry, BSc and Luc J. Martin, PhD
Université de Moncton
(Presented By: David Landry, BSc)

Introduction and Objectives: Sox genes encode a family of transcription factors characterized by a HMG box, which can bind and bend DNA through the consensus sequence (A/T)(A/T)CAA(A/T)G. Two members, Sry and Sox9, play important roles in male sex determination and differentiation in mammals. Leydig cells are essential for testosterone production in the testis. In these cells, the StAR protein allows cholesterol to enter the mitochondria and be converted to pregnenolone by the first steroidogenic enzyme Cyp11a1. Of the 20 Sox family members identified in vertebrates, several are expressed in gonads, including adult Leydig cells. Sox9 is expressed in steroidogenic cell lines, including MA−10 and R2C Leydig and Y1 adrenal cells. Interestingly, potential DNA regulatory elements for Sox members are present in promoter regions of steroidogenic genes, supporting that Sox9 might be involved in the regulation of steroidogenesis in Leydig cells. Our objective was to determine whether Sox9 regulates StAR and Cyp11a1 in Leydig cells and to better define its mechanism of action.
Methods: Mouse MA−10 Leydig cells were used in transfection and were harvested for total protein and total mRNA extractions. Protein quantifications were done by Western blot, whereas mRNA levels were determined by qPCR. Characterizations of Sox−dependent promoter activities of steroidogenic genes were done by transient transfections of MA−10 cells with StAR or Cyp11a1 promoter constructs and electrophoretic mobility shift assays (EMSA).
Results: Multiple potential Sox−dependent regulatory elements have been found in −1kb promoter regions for StAR and Cyp11a1, and these promoter constructs were activated 3 and 14 folds, respectively, by Sox9. Interestingly, PKA−dependent phosphorylation of Sox9 consistently reduced its transcriptional activity, as shown using transfection of a constitutively active PKA expression plasmid or 8Bromo−cAMP stimulations. Using 5' progressive deletion constructs for StAR (−843, −680, −515, −355, −72 bp) and Cyp11a1 (−888, −633, −427, −262 bp) promoters, regions important for Sox9−dependent activations were located between −680 and −515 bp for StAR and −88 and −633 bp for Cyp11a1.
Conclusion: Thus, our data identify Sox9 as a new regulator of steroidogenic genes expressions in Leydig cells. Future work will focus on post−translational modifications and protein−protein interactions involved in modulation of the transcriptional activity of Sox9 in Leydig cells.

ABSTRACTS

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EFFECTS OF METHOXYCHLOR AND ITS METABOLITE 2,2−BIS(P−HYDROXYPHENYL)−1,1,1−TRICHLOROETHANE ON HUMAN AND RAT 17A−HYDROXYLASE/17,20−LYASE ACTIVITY

Leping Ye, MD[1], Ren−Shan Ge, PhD[1] and Hui Li, MD, PhD[2]
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(Presented By: Leping Ye, MD)

Introduction: Exposure to methoxychlor, an agricultural pesticide, has been associated with reduced testicular androgen secretion. However, methoxychlor is converted to 2,2−bis−(p−hydroxyphenyl)−1,1,1−trichloroethane (HPTE) in the liver, which then acts as its biologically active metabolite. Both methoxychlor and HPTE have been credited with estrogenic properties and have a weak anti−androgenic activity. However, the exact mechanisms of steroidogenic enzyme inhibition remain to be clarified.
Methods: In the present study, human and rat testis microsomes were employed to investigate the inhibitory activities of methoxychlor and HPTE on 17α−hydroxylase/17,20−lyase (CYP17A1) activity. The CYP17A1 enzyme is critical for androgen biosynthesis and catalyzes conversion of progesterone into androstenedione.
Results: The results demonstrated that HPTE directly inhibited human and rat CYP17A1 activity, while methoxychlor had no effects on enzyme activity even at a concentration of 100 μM. The IC50 values of CYP17A1 for HPTE inhibition were 1.13 ï‚± 0.10 μM (human) and 6.87 ï‚± 0.13 μM (rat), respectively. When HPTE was incubated with intact rat immature Leydig cells, it also inhibited CYP17A1 activity with an IC50 value of 6.29 ï‚± 0.1 μM. Results of enzyme inhibition studies were supported by the observation that HPTE inhibited luteinizing hormone−stimulated 5α−androstane−3α, 17β−diol and testosterone secretion by immature Leydig cells with IC50 values of 6.61 ï‚± 0.03 and 3.78 ï‚± 0.003 μM, respectively.
Conclusion: The mode of action of HPTE on CYP17A1 activity was determined to be uncompetitive with the substrate progesterone. The reported suppression of androgen secretion by methoxychlor is presumably associated with inhibition of steroidogenic enzyme activity and has implications for endocrine function of the testis.

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EXPRESSIONS OF SOX5 AND SOX13 TRANSCRIPTION FACTORS ARE INCREASED IN TESTICULAR LEYDIG CELLS OF RODENTS DURING POST−NATAL DEVELOPMENT

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Université de Moncton
(Presented By: Mikella A. Daigle, BSc)

Introduction and Objectives: Members of the SRY−related HMG box (Sox) transcription factor family are proteins that have been conserved during the evolution of vertebrates. Sox members are expressed in numerous tissues and regulate a variety of developmental stages. Indeed, Sry upregulates Sox9 during sex determination and testes differentiation of the embryo. In post−natal testes, members of the Sox family, such as Sox5, Sox6, Sox8, Sox9 and Sox17, have been characterized. However, expressions of members of this family of transcription factors have never specifically been shown in adult Leydig cells. These cells supply testosterone necessary for the onset and maintenance of spermatogenesis. The objectives of this research are to locate and determine the expression profiles of two SoxD members, Sox5 and Sox13, in post−natal mice testes at different developmental stages, as well as to identify their expression in rodent Leydig cell cultures.
Methods: mRNA and protein quantifications of Sox5 and Sox13 from whole mice testes at three different ages (33 days, 8 weeks and 7 months) as well as in MA−10, R2C and primary cell cultures stimulated with 8Bromo−cAMP were done using quantitative qPCR and Western Blots, respectively. Immunohistochemistry was used to locate Sox5 and Sox13 protein expressions from whole mice testes at the same three developmental stages.
Results: Sox5 and Sox13 mRNAs and proteins have been characterized in MA−10, R2C and primary cell cultures, as well as from whole testes from 33 days, 8 weeks and 7 months old mice. Their expressions were independent of 8Bromo−cAMP stimulation. Using immunohistochemistry of mice testes, Sox5 and Sox13 expressions were confirmed to be located and to increase according to post−natal development of Leydig cells.
Conclusion: To our knowledge, this is the first study showing the presence of Sox5 and Sox13 transcription factors in adult Leydig cells. These proteins may regulate multiple functions of these cells, such as steroidogenesis important for puberty and spermatogenesis. However, their role and mechanisms of actions in post−natal testes remain to be investigated.

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DEHYDROEPIANDROSTERONE ANTAGONIZES SURGERY STRESS−INDUCED SUPPRESSION OF TESTOSTERONE PRODUCTION IN MALE RATS

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(Presented By: Han Lin, PhD, MD)

Introduction: Leydig cells secrete the steroid hormone testosterone, which is essential for male fertility and reproductive health. Stress increases the secretion of glucocorticoid (corticosterone, CORT, in rats) that decreases circulating testosterone levels in part through a direct action on receptors (GR) in Leydig cells. Intratesticular CORT level is dependent on oxidative inactivation of glucocorticoid by 11β−hydroxysteroid dehydrogenase 1 (11β−HSD1) in Leydig cells.
Methods: In the present study, we investigated the time−course changes of steroidogenic gene expression levels after acute immobilization stress in rats and the possible mechanism of dehydroepiandrosterone (DHEA) that antagonizes it.
Results: The serum CORT levels were significantly increased after 1, 3 and 6 hours of surgery−induced stress, while the serum testosterone levels were significantly decreased starting at 3 and 6 hours after stress. The 3−hour−surgery stress also decreased Star, Hsd3b1 and Cyp17a1 expression levels. Doses of 5 and 10 mg/kg DHEA were administered orally to adult Sprague Dawley rats 1 minute before surgery stress, and it antagonized surgery−mediated reduced testosterone level and the expression of Star, Hsd3b1 and Cyp17a1. DHEA was found to modulate 11β−HSD1 activities by increasing its oxidative activity and decreasing its reductive activity thus decreasing the intracellular CORT levels in the Leydig cells.
Conclusion: In conclusion, DHEA protects Leydig cell function from stress via modulating 11β−HSD1 activity.

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IMPROVING THE FERTILITY OF DOG

Gamal El−Amrawi, Professor
Alexandria University
(Presented By: Gamal El−Amrawi, Professor)

Methods: Semen quality of 22 German shepherd dogs was examined for 6 weeks before and after L−Carnitine supplementation (2 gm/dog for 2 weeks). Two ejaculates were collected by digital manipulation from each dog. The first, second and third fractions of the ejaculate were collected into 3 separate plastic test tubes via glass funnels and semen quality were assessed in the second fraction. The dogs were rested for 45 to 75 minutes (63.4±2.5 minutes) before semen collection (second ejaculate) and the same evaluation was repeated. Blood samples were collected from all dogs before semen collection for assessment of LH and testosterone concentrations.
Results: The results revealed that the second ejaculate had significantly lower values than the first one in both occasions. Semen characteristics in both the first and second ejaculates increased after L−carnitine supplementation, although values of the second ejaculates were still lower than the first one. LH and testosterone concentrations increased in the L−carnitine supplemented dogs.
Conclusion: It could be concluded that L−carnitine supplementation increased the libido and the quality of semen ejaculates in dogs.

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PREVALENCE OF BONE DENSITY DEFICIENCIES IN MEN PRESENTING FOR HYPOGONADISM TREATMENT: DO WE NEED TO WORRY?

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[1]Albany Medical College; [2]albany medical college; [3]Urological Institute of North Eastern New York
(Presented By: Igor Sorokin, MD)

Introduction and Objective: Hypogonadism is a known risk factor in men with osteoporosis. The prevalence of hip osteoporosis in men with total testosterone deficiency (<300ng/dL) is 4.3%. Therefore, it is recommended that baseline bone mineral density (BMD) studies be obtained in this population. The urologist is referred a unique population of men with varying durations of hypogonadism in various age groups with extremes of sex hormones. Our objective was to identify the rate of osteopenia and osteoporosis and the predictive risk factors associated with low BMD scans in the selective population that is referred to a Urologist.
Methods: A retrospective review of 95 consecutive patients with clinical hypogonadism (both symptoms and biochemical testosterone deficiency <300ng/dL) had BMD scans performed on a single Dual−energy X−ray Absorptiometry DEXA machine (Hologic 4500). Osteopenia was defined as a femoral neck, total hip, or total spine BMD T−score between −1 and −2.5. Osteoporosis was defined as a BMD T−score of −2.5 or less. Duration of hypogonadism was defined as time from 1st laboratory value noting low testosterone to BMD scan. Median testosterone and estradiol values were obtained from diagnosis of hypogonadism to BMD scans. Univariate and multivariate analysis were performed to determine the predictive risk factors of an abnormal BMD scan.
Results: The mean ± SD age of our cohort was 49.9 ±13.5 years. Median duration of hypogonadism was 10 months. The median initial testosterone at diagnosis and last testosterone before BMD scan was 179 ng/DL and 208 ng/DL, respectively. We found normal BMD in 51/95 patients (54%), osteopenia in 36/95 (38%), and osteoporosis in 8/95 (8%). On univariate analysis, age (OR 1.04, 95% CI 1.01−1.07, p=0.018) and smoking history (OR 5.2, 95% CI 2.107−12.5, p<0.001) were the only 2 significant factors associated with abnormal BMD scans. Sex hormones, Body mass index (BMI), hypertension, diabetes, or duration of hypogonadism were not predictive of abnormal BMD scans.
Conclusion: There is a very high rate of osteopenia and osteoporosis in male patients with hypogonadism referred to a urologist. We found no single testosterone value <300 ng/dL that would be predictive of an abnormal bone scan. This study reiterates the importance of obtaining BMD scans on all male patients with clinical hypogonadism.

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CHRONIC CYCLOPHOSPHAMIDE TREATMENT AFFECTS GENE EXPRESSION IN PACHYTENE SPERMATOCYTES AND ROUND SPERMATIDS

Anne Marie Downey, Barbara Hales, PhD and Bernard Robaire, PhD
McGill University
(Presente By: Anne Marie Downey)

Introduction and Objective: As the numbers of men of reproductive age who survive cancer and wish to father children increase, it is becoming increasingly important to understand the effects of chemotherapy on male germ cells and reproductive outcome. Previous studies from our laboratory have shown that paternal exposure to cyclophosphamide, a chemotherapeutic agent and immunosuppressant, has detrimental effects on sperm quality and progeny outcome. How cyclophosphamide affects the developing germ cells and how they respond to this insult remain unresolved. The purpose of this study is to test the hypothesis that cyclophosphamide affects gene expression in pachytene spermatocytes and round spermatids.
Methods: Adult Sprague−Dawley male rats were gavaged with cyclophosphamide (6 mg/kg) or saline, 6 days/week for 4 weeks. Pachytene spermatocytes (n=5) and round spermatids (n=6) were collected by unit gravity sedimentation using the STA−PUT method. Total RNA was isolated and mRNA expression was profiled using whole genome gene expression microarrays. Data was analyzed with Genespring 12.0 and Pathway Studios software.
Results: In pachytene spermatocytes 252 transcripts were significantly changed by more than 1.5 fold: 97 were up− and 155 down−regulated, compared to controls. In round spermatids, 230 transcripts were significantly changed by more than 1.5 fold: 124 were up− and 106 down−regulated, compared to control. Differential expression of transcripts coding for genes involved in the DNA damage response and the regulation of cell death was observed in both cell types. In pachytene spermatocytes, the expression of 3 genes involved in base and nucleotide excision repair pathways was altered, while in round spermatids, the expression of genes involved in base excision, homology directed and DNA alkylation repair was altered. In pachytene spermatocytes, the expression of many transcripts coding for genes involved in the tumor necrosis factor receptor 1 (TNFR1) pathway was altered. In contrast, transcripts coding for genes involved in the TNFR1 pathway were not affected by drug treatment in the round spermatids.
Conclusion: These results suggest that chronic cyclophosphamide treatment results in different DNA damage and survival responses in pachytene spermatocytes and round spermatids. The altered ability of these cells to respond to DNA damage and survive may lead to damaged mature spermatozoa.
These studies are supported by CIHR.

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ACTION OF RESVERATROL ON THE REPRODUCTIVE PARAMETERS OF LATE PUBERTAL RATS TREATED WITH ANTI−CANCER AGENTS (BEP PROTOCOL MODIFIED), FROM PERIPUBERTY

Flavia Macedo de Oliveira Neves, PhD Student, Vanessa Vendramini Vilela, Collaborator and Sandra Maria Miraglia, Advisor
Federal University of Sao Paulo − UNIFESP − Brazil
(Presented By: Flavia Macedo de Oliveira Neves, PhD Student)

Introduction and Objective: The incidence of testicular cancer in 15 to 35−years old men has increased over the last 50 years. Protocols including the co−administration of bleomycin, etoposide and cisplatin (BEP) have been effective, rising patient survival. However, deleterious effects on the reproductive health of patients have been reported. Resveratrol (R) is an antioxidant fitoalexin that shows anti−apoptotic properties. Our aim is to study the potential protection of resveratrol against the side effects on reproduction caused by the BEP administration from peripuberty.
Methods: From the 36th day post partum (dpp) rats were resveratrol−treated (gavage) with a daily single dose of 300mg/kg per 5 days and subsequently (from 41st dpp on) submitted to co−administration of R and BEP (R−BEP group) applied for three consecutive weeks: etoposide (3.50mg/kg) and cisplatin (0.70mg/kg) for 5 consecutive days/week and bleomycin (0.35mg/kg; every 2nd day of each week); all drugs were injected by intraperitoneal (ip) route. Three other groups were solely treated with: 1− BEP (BEP group), 2− resveratrol (R group), and 3− carboxi−methyl−cellulose (vehicle of resveratrol by gavage) plus saline 0.9% (ip route; SC− Sham Control group). Testis and epididymis biometric parameters, histopathological analysis, morphometric and stereological testicular parameters, spermatic evaluation and sperm mitochondrial activity were investigated. HTM−IVOS motility analyser was utilized.
Results: Rats of BEP and R−BEP groups showed reduction of: body weight, epididymal and testis weights, testis morphometric parameters and germ cell depletion. A higher frequency of sperm anomalous forms in epididymis cauda was observed in the BEP and R−BEP groups. The BEP group presented a higher frequency of TUNEL−positive germ cells and a lower mitochondrial activity when compared to the R−BEP group. Although the sperm motility characteristics were altered in BEP and R−BEP groups, the parameter reflecting sperm flagellar beating was only altered in BEP group. Sex hormone dosage, testis oxidative stress and sperm DNA fragmentation are being investigated. The reduction of apoptosis in germ cells, the improvement of sperm mitochondrial activity and of sperm flagellar beating in R−BEP group point out to a reduction of the reproductive damage caused by BEP treatment.
Conclusion: Additional studies are being performed to better clarify the potential protective action of R against the deleterious effect of this treatment.

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FETAL CYCLOPHOSPHAMIDE EXPOSURE INDUCES TESTICULAR CANCER AND REDUCES SPERMATOGENESIS IN MICE

Gunapala Shetty, PhD, Ana Luiza Drumond, PhD, Paul Comish, MS, Angabin Matin, PhD and Marvin Meistrich, PhD
University of Texas M.D. Anderson Cancer Center
(Presented By: Gunapala Shetty, PhD)

Introduction and Objectives: There has been a 3−fold increase in the incidence of testicular germ cell tumors (TGCTs) and a 50% decline in sperm counts over the past 60 years. Both these adverse outcomes have been suggested to be the results of prenatal exposure to environmental agents. Previously we showed that fetal exposure to radiation induced testicular germ cell tumors (TGCT) in 129.MOLF−congenic−L1 (L1) mice, which are genetically susceptible to testis cancer, and also reduced spermatogenic function in the testes that did not develop cancer.

Methods: Here we tested whether fetal exposure to a gonadotoxic and carcinogenic chemical could also have the same effects in L1 mice and whether it could also induce tumors in standard strains of 129 mice. We chose cyclophosphamide (CP), an alkylating agent, because pregnant women currently being treated for breast cancer are exposed to it. CP was given to pregnant L1 and 129 mice at 7.5 mg/kg on embryonic days 10.5 and 11.5.
Results: The treatments dramatically increased the TGCT incidence to 80% in the male offspring of L1 mice (control value 33%) and to 28% in the offspring of 129 mice (control value, 2%). The weights of testes with tumors in CP−treated L1 mice were higher than those in controls, indicating that treatment induced multiple foci of initiation sites in each testis. Furthermore, in utero CP exposure produced a loss of germ cells as testes weights of both 129 and L1 offspring were significantly reduced to ~70% of the respective controls and atrophic tubules were observed in about 30% of the testes. All the results obtained with CP treatment in both lines of mice are similar to those observed after irradiation.
Conclusions: The results obtained here suggest that i) DNA damage seems to be a common mechanism leading to induction of testicular cancer; ii) the susceptibility to induction of testis cancer by external agents in individuals of different genetic susceptibility is proportional to the spontaneous incidence; and iii) the male fetus of women exposed to DNA damaging chemotherapeutic agents during pregnancy might have reduced spermatogenesis and an increased risk of developing testis cancer.

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EFFECTS OF EUCOMMIAE CORTEX (EC) ON SPERM COUNT AND MOTILITY PARAMETERS IN MALE MICE

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Department of Prescriptionology, College of Korean Medicine, Kyung Hee University
(Presented By: Mun Seog Chang, PhD)

Introduction and Objective: The process of sperm cell development is usually represented the spermatogenesis. This process explained as undergoing mature mitotic and meiototic divisions and a metamorphic change (spermatozoa). The cyclic AMP response element modulator (CREM) is a crucial role of the differentiation of round spermatids into mature spermatozoa and the expressions of many important post−meiotic genes. Eucommiae Cortex (EC), a medicinal herb, was widely used to treatment for impotence, male infertility in traditional medicine. The purpose of this study was to investigate the effects of EC on the male reproductive system and the CREM expressions in cyclophosphamide (CP)−induced mouse.
Methods: We performed Real time−PCR and western blot analysis for CREM expression and examined sperm parameters.
Results: CREM mRNA level was analyzed by real time PCR in this study in which100 mg/kg of CP and 500, 1000 mg/kg of EC treated group were significantly down regulated than CP treated group. Also, the protein levels of CP with 100 and 1000 mg/kg of EC treated groups were increased than CP treated group as well, but there was no significance.
Conclusion: Following the result data, this study suggest that Eucommiae Cortex treatment reduce the reproductive toxicity in male reproductive system by increasing CREM gene expression and protein biosynthesis in mouse testis.

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LATE REPRODUCTIVE ANALYSIS OF RAT MALE OFFSPRING EXPOSED TO NICOTINE DURING PREGNANCY AND LACTATION – FINAL PART

Mayra Miranda−Rodrigues, Masters Student, Camila C. Paccola, Collaborator, Samara U. Oliva, Collaborator and Sandra M. Miraglia, Advisor
Federal University of Sao Paulo − UNIFESP − Brazil
(Presented By: Mayra Miranda−Rodrigues, Masters Student)

Introduction and Objectives: Around 1/3 of the world population smokes and 10.4% of pregnant women report smoking during pregnancy in United States. Nicotine (Ni), present in cigarettes, reaches the maternal milk and cross the placental membrane. It inhibits steroidogenesis, suppresses testosterone secretion in adult male rats and causes erection dysfunction, testicular atrophy and infertility. Previously, we observed that Ni, when injected in rats during whole pregnancy and lactation periods, provokes, in the progeny, late morphofunctional alterations of Leydig cell, body weight raise in adulthood (90 days postpartum−dpp) as well as an evident seminiferous epithelium injury. Aiming to investigate whether the spermatogenic damage previously observed in 90dpp progenies from pregnant and lactating Ni−exposed rat dams are maintained or whether it is worsen in older rats, we analyzed the morphological testicular alterations after up to two complete periods of spermatogenesis (53 days each), spermatic parameters and sperm DNA fragmentation.
Methods: Pregnant and lactating rats were Ni−exposed (2mg/Kg/day) through an osmotic minipump implanted at the first day of pregnancy and replaced after birth. Absolute Control (no minipump implanted) and Sham Control (minipump implanted without Ni) groups were established. The offspring was killed at 143dpp and 196dpp.
Results: Significant alterations of morphometric and stereological testicular parameters were not observed in Ni−exposed rats. The testicular histopathological analysis showed small intraepithelial vacuolization and germ cell desquamation in Ni−exposed rats. Testicular concentration of step 19 spermatids, daily sperm production, concentrations and transit time of the sperm in the head/body and cauda of the epididymis did not also show significant changes. The plasmatic and intratesticular levels of cholesterol and testosterone were not significantly changed among the groups in both ages studied. However, the offspring from Ni−exposed dams exhibited a higher frequency of morphologically abnormal sperms as well as lower sperm motility in comparison with both control groups. In addition, the Ni−exposed groups showed a significant reduction of the sperm mitochondrial activity and an increased sperm DNA fragmentation (Comet Assay).
Conclusion: These results indicate a late reproductive damage in the male progenies provoked by maternal Ni−exposure, related to the decrease of the sperm quality.

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MOLECULAR ALTERATIONS IN SPERM ARE SENSITIVE INDICATORS OF TESTICULAR DYSFUNCTION

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(Presented By: Linnea Anderson, MSc)

Introduction: Traditional endpoints used to measure male reproductive toxicity in humans, including semen and hormone analysis, are insensitive and unreliable; those used to monitor toxicity in animal studies, while sensitive, are not easily translatable to humans. It is therefore necessary to develop sensitive and reliable molecular biomarkers of testicular injury that can be used to both monitor human reproductive function and compare animal studies with human exposures.
Objectives: The aim of this research is to use exposures to model testicular toxicants to identify sperm molecular alterations in rats, and to examine these alterations in sperm from clinically fertile and subfertile men.
Methods: Adult male rats were exposed to cyclophosphamide (CPP) for 12 weeks (1.4, 3.4, or 5.1 mg/kg/day p.o.) or 12 weeks plus an additional post−exposure recovery period of 12 weeks (5.1 mg/kg/day p.o.) as a model of germ cell toxicity. Standard reproductive endpoints were examined to assess testicular injury; in particular, germ cell apoptosis and spermatid head retention were quantified as sensitive markers of damage. mRNA isolated from cauda epididymidal sperm was analyzed for toxicant−induced alterations using a genome−wide microarray, then significant and robust alterations were further examined using qRT−PCR arrays.
Results: CPP produced dose−dependent testicular injury that resolved after a 12−week recovery period. The levels of injury correlated with specific changes in transcript abundance, indicating a utility for these mRNAs as translatable biomarkers for male reproductive dysfunction.
Conclusions: We have previously identified mRNA transcripts that are sensitive to low doses of Sertoli cell toxicants, and have now identified a panel of transcripts that sensitively identifies testicular dysfunction induced by germ cell toxicants. These transcripts will be examined in additional exposure settings, as well as both fertile and subfertile men to continue to validate the relevance of these alterations.

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NETWORK ANALYSIS OF REDOX MEDIATED PROTEIN−PROTEIN INTERACTIONS IN SPERMATOZOA

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Introduction and Objectives: Growing evidence suggests that the cellular redox status regulates sperm function and sperm quality. Defective sperm function is the major single defined cause of infertility in humans. Redox imbalance can cause positive responses such as activation and negative responses such as inhibition and deterioration in lipid membrane and DNA packaging in spermatozoa. However, post−translational modifications in proteins are the most abundant damages caused by oxidative stress in spermatozoa. Recently, proteomic studies have started to build the protein expression datasets for human sperm, however, interactions between human sperm signaling pathway proteins and redox status in spermatozoa yet to be discovered. Predicting redox mediated protein− protein interactions (PPIs) in spermatozoa is important for the transcriptionally silenced spermatozoa and it will help identify the key regulators and their interactors that can serve as drug targets to restore redox balance and can be used for prioritization of candidate male infertility related genes. Aim of this study was to perform network analyses on manually curated and experimentally supported interactomes from different repositories and databases such as The DIPTM Database, MatrixDB, BioGRID, MINT, and IntAct. UniProtKB accession numbers for each protein were used as global protein identifiers.
Methods: Four types of PPIs were categorized as physical, regulatory, genetic interactions and similarity relations. The PPI network predictions and maps were cross−referenced with STRING when possible. Redox regulated proteins including thioredoxins (Trx), peroxiredoxins (Prdx), glutathione peroxidases (Gpx) and other peroxidases, and the proteins involved in ROS metabolism were selected as focus nodes. At 0.40 reliability score, the network analyses were performed for 84 oxidative stress proteins against each dataset where possible. Mentha interactome browser was used for the network analyses.
Results: Antioxidant proteins, proteins involved in superoxide and oxidative stress response proteins were among the highest reliability scoring nodes pointing highest number of interactions to these proteins.
Conclusion: Although measurements of PPIs tend to be noisy and incomplete, predictive network analysis of redox mediated sperm interactome would be helpful guide to better understand the signaling cascades in spermatozoa and for prioritization of candidate male infertility related genes for developing non−hormonal male contraceptives.

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MAPPING THE SPERM MEMBRANE PROTEIN INTERACTOME

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(Presented By: Matthew Marcello, PhD)

Introduction: The interaction and organization of proteins in the sperm membrane are important for recognition of and fusion with the egg. We have determined the interactions between all known sperm membrane proteins in a model system for reproduction, the nematode Caenorhabditis elegans. Identification of the interactions between sperm membrane proteins will improve our understanding of and ability to characterize defects in these processes.
Methods: To identify interacting proteins, we are performed pair−wise split−ubiquitin yeast two−hybrid analysis of the full−length gene products.
Results: Our analysis revealed novel interactions between sperm membrane proteins known to have roles in spermatogenesis, spermiogenesis, and fertilization. For example, we found that a protein known to play a role in sperm function during fertilization, SPE−38 (a predicted four pass transmembrane protein), interacts with proteins necessary for spermiogenesis and spermatogenesis.
Conclusion: These novel interaction pairings will provide the foundation for understanding membrane protein interactions during spermatogenesis, spermiogenesis, and sperm function during fertilization. The interactome provides a more comprehensive view of sperm membrane protein interactions and the rationale for investigating previously unrealized connections.

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COMPARATIVE ANALYSIS OF MACAQUE AND HUMAN SPERM PROTEOMES: INSIGHTS INTO SPERM COMPETITION

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(Presented By: Tao Zhou)

Introduction and Objectives: Sperm competition is defined as sperms from different males compete for the chance of fertilization in the reproductive tract of a single female. Macaques are promiscuous and humans are monogamous, thus male macaques have higher pressure of sperm competition than male humans. Sperm competition has been a selective force that shaped many male reproductive features. Previous studies have found that macaques have larger testis size and greater sperm motility compared to humans. Our objective is to explain the differences of phenotypes between macaque and human under sperm competition at the protein level.
Methods: We firstly constructed macaque and human sperm proteomes using liquid chromatography−tandem mass spectrometry. We then detected the positively selected genes specifically on the branch of macaque based on branch−site likelihood method. Bioinformatics method was used for mining the biological and medical significance of positively selected genes. We further compared the ultrastructural differences of the mid−piece between macaque and human sperms to provide evidence for our findings using transmission electron microscopy.
Results: We identified 204 positively selected sperm genes specifically on the branch of macaque. These genes are highly associated with mitochondria and axoneme which directly drive sperm motility. We further showed that macaques have more mitochondrial gyres in mid−piece of sperm than humans. Taken the 175 human sperm orthologs of macaque sperm positively selected genes as the molecular targets of relaxation in humans, we found that ciliary motility disorder is the most significant enriched human disease. Using the information of mouse phenotypes, we also showed that the relaxation of sperm competition may be associated with poor sperm motility.
Conclusions: Our results explained the differences of phenotypes between macaque and human under sperm competition at the protein level, and also provided resources for the analysis of male infertility. We found that sperm competition has impacts on genes associated with energy production and molecular motor which are directly drive sperm motility. Sperm in humans with low motility or genetic disorders may also have higher opportunity for inheritance than in macaques. Thus we speculated that the poor sperm motility of humans may be associated with the relaxed selective pressure during evolution.

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TRANSCRIPTIONAL PROFILING OF HYPOXIA PATHWAY GENE EXPRESSION IN THE RAT TESTIS FOLLOWING P. AERUGINOSA LIPOPOLYSACCHARIDE−INDUCED INFLAMMATION

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(Presented By: Genevieve Fasano)

Introduction and Objectives: Inflammation of the male reproductive tract by bacterial infections is known to suppress androgen production and can result in infertility. Research on antimicrobial properties of the testis has advanced an understanding of specific genes and proteins involved in the detection and clearance of invading microbes. We have shown that hypoxia−inducible factor−1 (HIF−1), considered the master regulator of oxygen homeostasis, increases following lipopolysaccharide (LPS)−induced inflammation suggesting roles for hypoxia regulated genes in inflammatory responses of the testis. We hypothesize that antimicrobial protection of the testis is achieved through both classic inflammatory pathways and hypoxic pathways. The goal of this work is to determine the effects of LPS−induced inflammation on gene expression pathways of the rat testis. The objective of this project was to identify hypoxia pathway genes that are up−regulated or down−regulated following LPS administration and to determine the role of these genes in response to inflammation.
Methods: Inflammation in rats was accomplished via i.p. administration of LPS from P. aeruginosa (5 mg/kg body weight) for 3 or 6 hours (n = 6−7 animals/time point). RNA was isolated from testes and cDNA synthesized for analysis by qPCR. The RT2 Profiler™ PCR Array Rat Hypoxia Signaling Pathway (Qiagen) was used to evaluate expression of 91 genes involved in hypoxia pathways.

Results: Array results demonstrated that 9 genes (Adm, Angptl4, Egr1, Fos, Ier3, Nfkb1, Pgf, Serpine1, Slc2a1) were up−regulated after 3 hours of LPS−induced inflammation and expression of 3 genes (Angptl4, Egr1, Serpine1) remained elevated after 6 hours. In silico analysis of LPS−stimulated genes indicates that these transcripts are predominantly expressed in supporting cells of the testis (Sertoli, myoid, and Leydig cells) and not in developing germ cells. Egr1, Fos, Ier3, and Pai1 are known target genes for the transcription factor NF kappa B.
Conclusions: A subset of hypoxia−sensitive genes were up−regulated following LPS treatment and are expressed in supporting cells of the testis. While some genes are known targets of NF kappa B, their functions will be studied to propose molecular mechanisms of antimicrobial responses in the testis. Future experiments will investigate gene expression in the inflammatory pathway following LPS treatment.
Funding: Independent College Fund of NJ–Merck Undergraduate Science Endeavors Scholarship.

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OLIGOZOOSPERMIA TRANSCRIPTOME PROFILE AND GENE CANDIDATE DISCOVERY IN SEMEN FROM INFERTILE MALES

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(Presented By: Alexander N. Yatsenko, MD, PhD)

Introduction: Male infertility is a common and complex health condition. About 20% of infertile men suffer from reduced sperm count, or oligozoospermia. In many cases, a genetic factor may contribute to their infertility, though the clinical ability of detecting these abnormalities is limited. Sperm RNA could be powerful tool in determining abnormal gene expression and the viability of germ cells in fertility clinics.
Methods: We isolated high quality RNA from severe oligozoospermic (OZ) and normozoospermic (NZ) patients semen. RNAs from 6 patients with highly uniform semen parameters were pooled into 4 groups. Two experimental and 2 control groups of samples were used for analysis: Exp 1, severe OZ 2−6 $x10^6$ sperm/ml; Exp 2 mild OZ 10−12 $x10^6$ sperm/ml; Control 1, NZ 64−77 $x10^6$ sperm/ml; NZ 115−155 $x10^6$ sperm/ml. In this study, we performed semen RNA−sequencing (RNA−seq) to determine the transcriptome profiles of both OZ and NZ.
Results: After rRNA reduction and libriray construction by random primer cDNA synthesis, we obtained an average of 52 million paired 75 bp sequence reads with ~63x coverage. Sequence analysis, by Super−Trascript level coverage, revealed 17,309 total transcripts in NZ samples and 21,098 in OZ patients uniquely mapped to reference genome. Gene expression data analysis of differential transcript quantities between samples revealed 214 transcripts with reduced amount in OZ and 216 with increased amount in the OZ. Among the down regulated transcripts in OZ, 9% (20/214) were previously implicated in gene knockout mouse models displaying male factor infertility. An additional 48% (102/214) of transcripts with reduced abundance in OZ, shown high testis expression, suggesting a role in male reproduction. Pathway analysis revealed downregulation of transcription, RNA binding, cell division, energy metabolism, apoptosis, and early embryonic maintenance. Based on these results we identified 64 candidate genes for male infertility.
Conclusion: We propose that these RNAs, in the unique transcriptome profile of oligozoospermic semen, could be of high clinical utility as a powerful diagnostic tool in assessing idiopathic male infertility.

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WHEREAS ALL CASES OF FAILED FERTILIZATION WITH CONVENTIONAL OOCYTE INSEMINATION WITH NORMAL SPERM ACHIEVE GOOD FERTILIZATION RATES WITH ICSI ONLY HALF WITH NORMAL BINDING HAVE GOOD FERTILIZATION RATES

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(Presented By: Jerome Check, MD, PhD)

Introduction and Objective: Failure to fertilize any oocytes despite conventional oocyte insemination with sperm that appear normal can be related to failure of the sperm to bind to the zona pellucida or failure to induce post−binding events leading to oocyte activation. The problem can be related to a sperm defect despite the normal appearance by standard semen parameters (including absence of anti−sperm antibodies) or an oocyte defect. One objective of this study was to determine what percentage of the time is failed fertilization related to failure of sperm to attach to the zona pellucida. A second objective was to compare the relative efficacy of ICSI to overcome fertilization failure according to cause of failed fertilization, i.e., failure of sperm binding or post−binding events.
Methods: Retrospective review of all IVF cycles evaluating all IVF cycles where there was failed fertilization following conventional insemination with normal appearing sperm was performed. A minimum of 3 oocytes retrieved was required. ICSI was offered in a succeeding IVF cycle. Fertilization rates with ICSI were then compared according to reason for failed fertilization−sperm binding or failure to activate the oocyte.
Results: 12 cases of failed fertilization were identified over a 13 year period in 12,448 IVF cycles. 6 of 12 were related to very few or no sperm attached to the zona pellucida. 2 cases with zona binding defects who failed to fertilize any of 16 inseminated oocytes shared a pool of oocytes with 2 other couples. The 2 male partners of these other couples fertilized 11/15 (73.3%) of the oocytes with conventional stimulation suggesting sperm receptor defect for zona protein (ZP) 3 or ZP4 rather than mutated ZP3 or ZP4 in the oocyte. ICSI negated the sperm binding defects with all 6 couples showing >50% fertilization with a total percentage of 73% (60/82). ICSI was not as effective with failed fertilization with normal sperm binding with 2 couples out of 5 (one did not try IVF again) showing failed fertilization (0/7) or poor fertilization (12.5%, 1/8). The other 3 had very good fertilization rates of 88.8% (16/18).
Conclusions: Failed fertilization following conventional oocyte insemination with sperm with normal semen parameters is uncommon. Failure of sperm binding accounts for 50% of the cases and is corrected by ICSI. ICSI by attaining a rapid calcium influx overcomes phase I but not phase II oocyte activation defects.

ABSTRACTS

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SPERM WITH LOW HYPO−OSMOTIC SWELLING (HOS) TEST SCORES MAY BE A RARE CAUSE OF RECURRENT MISCARRIAGE

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Introduction and Objective: For over 30 years our group demonstrated that males with consistently low HOS test scores <50% do not achieve live pregnancies by either intercourse or conventional IUI. IVF with conventional oocyte insemination leads to normal fertilization of normal morphologic embryos but they do not implant and thus do not result in clinical pregnancies. However, ICSI or pre−treating the sperm with chymotrypsin−galactose prior to IUI allow normal pregnancy rates. There has been one study that suggested that low HOS can be a cause of miscarriage (Buckett et al., Fertil Steril, 1997). A prospective observational study was initiated to either confirm or refute the aforementioned study.
Methods: Our staff was alerted to report any women whose husband had a low HOS test and seemed to achieve a pregnancy without IVF with ICSI or IUI with chymotrypsin treated sperm.
Results: 5 years from Buckett's article a case of a woman who achieved a pregnancy despite a low HOS test was found but it was an ectopic. 11 years later we found a case of low HOS test and miscarriage. One couple had a live birth when the female partner was age 35 and the male partner was 51. Subsequently she had a miscarriage 6 and 10 months after delivery and then another miscarriage 3 years after delivery followed by an ectopic pregnancy 5 months later. She consulted us for recurrent miscarriage. A semen analysis with HOS test was suggested but he procrastinated. She conceived naturally again following taking letrozole for a follicular maturation defect with vaginal progesterone in the luteal phase. Unfortunately she had another miscarriage. Three months after the last miscarriage the male partner produced a semen specimen. It had low volume of 0.7mL with a concentration of 175x106/mL but only 15% motility and an HOS test of only 36%. Two subsequent semen analyses 1 week and 6 months later continued to show low % motility (6% and 8%) and low HOS test scores (37 and 30% respectively).
Conclusions: The last pregnancy and miscarriage was very likely achieved by a sperm specimen with a low HOS score. Possibly the previous ectopic or other miscarriages could have been related to the low HOS test scores. The implantation defect related to oocytes fertilized by sperm with low HOS scores rarely leads to a pregnancy, but if one occurs, it is likely to end in miscarriage or an ectopic.

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THE ROLE OF JUSTICIA GENDARUSSA BURM.F., AS MALE CONTRACEPTION, ON BLOOD LIPID PROFILE

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(Presented By: Sri Musta'ina, Master)

Introduction and Objectives: Contraceptives used to control population must consider the aspect of safety, security and the effectiveness (trusted efficacy and its use can be interfere with the need) comfort (easy to use, does not interfere husband−wife relationship, can be received by the spouse), the nature of reversibility and avoid surgery (Albar, 1991; Lissner, 1994; Liu, 1998). Gendarusin A is a major component of Justicia gendarussa Burm. f. leaves that was reported to have antifertility effect by degrading activity of hialuronidase enzyme. One of the security aspects to note is its influence on the blood lipid profile, blood lipid profile is given one of the risk factors for the occurrence of disturbed heart function. This study is to investigate the effect of 70% etanol extract of Justicia gendarussa Burm.f. in blood profile lipid.
Methods: 21 healthy men according to preliminary laboratory examine is randomized and controlled by clinical trial to consume Justicia gendarussa Burm.f. (each capsule contain 450 mg 70% ethanol extract of Justicia gendarussa leaves that equal with 2,9 mg gendarusin A) once a day after breakfast for 30 days. Blood sample were obtained at day 0, day 15, day 30 and day 60 along drug and recovery period (30 day after stopping drug treatment. Blood Serum was analyzed using Roche Modular analytics SWA system treatment. Cholesterol screening is done by the method of Cholesterol Oxidase CHOD−POD. Determination of the levels of triglycerides is done by the Enzymatic method of Glycerol Blanking, Determination of LDL−cholesterol levels is by a method of Enzymatic end point (Homogenous direct Method). Data analysis with GLM Repeated Measure Anova method.
Results: The results obtained by comparing data from laboratory examines on day 0, day 15. day 30 and day 60. Its results showed that the mean value of total cholesterol ratio, triglyceride, HDL−cholesterol, and LDL−cholesterol between day 0, day 15, day 30 and day 60 showed no significance difference to the value of $\alpha = 0.05$.
Conclusion: 70% etanol extract of Justicia gendarussa Burm.f. in capsule did not alter blood lipid profile).

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INVESTIGATING THE SPERMICIDAL PROPERTIES OF NOVEL COMPOUNDS

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(Presented By: Ashley Robertson)

Introduction and Objectives: The number of unintended pregnancies in the United States is a concern, as there are a number of associated negative economic and health related consequences. A goal in the Family Planning topic of Healthy People 2020 is to prevent unintended pregnancies. One potential mechanism to reduce unintended pregnancies is through the development of novel spermicides. Spermicides currently available are effective; however, there have been recent concerns with their safety. The most common over−the−counter spermicide ingredient is nonoxynol−9 (N9). N9 is a nonionic surfactant with spermicidal properties. Other consumer products such as shaving cream, cleaning products, poison ivy ointment, and sports cream contain N9 as well. Although N9 is effective at killing sperm, studies have shown it has detrimental side effects. The United States Food and Drug Administration issued a new rule effective June 2008 which required warning labels to be placed on over−the−counter vaginal contraceptive products containing N9. The goal of this research was to investigate the spermicidal properties of novel compounds as potential spermicides.

Methods: Human semen samples were collected according to an IRB approved Human Subjects protocol. Whole semen was initially analyzed to determine volume of ejaculate, cell concentration, pH, cell morphology, and percent motility. Spermicidal assays were conducted using whole semen. Briefly, whole semen was incubated with various concentrations of N9 or novel compound at 37°C for a minimum of 5 minutes. Following incubation, the sample was gently mixed and a small aliquot was removed to microscopically determine percent motility, cell morphology, and cell concentration.
Results: Whole semen incubated with 1% N9 for 5 minutes showed 0% motility following treatment. A slight reduction in motility was noted with treatment of whole semen with 0.001% N9 for 5 minutes. Treatment of whole semen with novel compounds A13TEG and A7TEG showed a reduction in sperm motility.
Conclusion: N9 showed spermicidal properties below over−the−counter product concentrations. Treatment of whole semen with our novel compounds resulted in reductions in sperm motility. Previous studies from our lab have shown that these novel compounds are less toxic to HeLa cells grown in culture. Taken together, these experiments support the notion that our novel compounds show potential as useful spermicides.

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A MODEL FOR STUDYING PROTECTION FROM INFERTILITY AFTER CHEMOTHERAPY: CYCLOPHOSPHAMIDE (CYP) DECREASES METASTATIC LUNG MELANOMA FORMATION AND INCREASES GERM CELL APOPTOSIS IN MICE

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(Presented By: YanHe Lue, MD)

Introduction: Preservation of fertility in young patients with cancer after chemotherapy is important for their quality of life. We have demonstrated that humanin (HN), a mitochondria derived 24 amino acid peptide, could attenuate male germ cell apoptosis after CYP treatment in rodents. This leads to the question of whether synthetic HN, while protecting germ cells from apoptosis, might also inhibit cancer cell apoptosis after CYP treatment. \
Methods: To examine whether CYP treatment was able to simultaneously suppress metastatic lung tumor formation and induce germ cell apoptosis, we studied 16 young adult male mice. Four mice were used as control. Twelve mice were challenged intravenously with B16 murine melanoma cells (200,000 cells/mouse) expressing the firefly luciferase gene (B16−Fluc). Among these 12 tumor−bearing mice, 4 mice received no treatment, and 2 groups of 4 mice were treated with a single CYP i.p. injection (200mg/kg BW) either at 1 or 2wks after B16−Fluc injection. Mice were imaged by IVIS bioluminescent imaging at 1, 2 and 3wks after 16−Fluc cell injection to detect lung metastases. Once tumor burden was determined, all mice were sacrificed at the end of 3wks. The numbers of tumors in the lungs were counted under stereomicroscopy. Germ cell apoptosis was detected by TUNEL assay and quantified as the number of apoptotic seminiferous tubules per 100 tubules expressed as Apoptosis Index (AI).
Results: CYP treatment diminished tumor burden in the lungs compared to non−treated mice. Without changes in body weight, CYP treatment decreased the number of lung tumors (NT) significantly ($p<0.001$) at 1wk (NT: 4.01±2.58) and more dramatically at 2wks (NT: 1.25±1.26) compared to non−treated tumor−bearing mice (NT: 10.33±1.16). While decreasing lung tumor formation, CYP treatment significantly ($p<0.001$) decreased testis weight at both 1 (79.75±9.64mg) and 2wks (60.25±3.95mg) as compared to control (102.10±9.64mg) and non−treated tumor−bearing (105.32±3.65mg) mice. CYP treatment for 2wks significantly ($p<0.001$) increased germ cell apoptosis (AI: 37.41±2.33) in comparison with control (AI: 15.67±2.02) and non−treated tumor−bearing (AI: 16.41±1.17) mice.
Conclusion: We conclude that 1) CYP significantly decreases metastatic lung tumor formation and increases germ cell apoptosis in mice; 2) the Metastatic Lung Melanoma (MLM) mouse model can be utilized to study oncofertility; and 3) the MLM mouse model will allow studies of humanin's fertility protective action using the CYP model of chemotherapy.

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DEVELOPMENT OF MALE NON−HORMONAL CONTRACEPTIVES BY TARGETING LATE SPERMIOGENESIS

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(Presented By: Wei Yan, MD, PhD)

Introduction: Overpopulation and high unintended pregnancy rate highlight a critical need for next−generation contraceptives, which should be safer, more convenient, effective and affordable, and can fit the needs of both women and men at different stages of their reproductive lives, with different ethnic, cultural and religious backgrounds, and different economic status worldwide. However, no male non−hormonal pills are currently available. Based on the fact that functional disruptions of late spermiogenesis (after the onset of spermatid elongation) can lead to the production of deformed and/or non−functional sperm and thus male infertility, but rarely cause testis shrinkage, we specifically proposed in 2009 that late spermiogenesis−specific gene products are ideal targets for male non−hormonal contraceptive drugs/pills. SPEM1 is a protein exclusively expressed in elongated spermatids, and inactivation of Spem1 gene leads to male infertility in mice, which is due to deformed sperm characterized by heads bent back and wrapped by residual cytoplasm, and that Spem1−null sperm cannot develop vigorous and long−lasting progressive motility. To find a compound that can cause sperm deformation similar to that seen in Spem1−null sperm, we embarked on an extensive search for known drug candidates documented to cause sperm deformation as a side or toxic effect during preclinical testing or clinical trials.
Methods: After testing numerous such compounds, we found a natural compound purified from a Chinese herb can cause sperm head−bent−back deformation in a way almost identical to that seen in Spem1−null mice. We, therefore, named the compound spermatodeformin 1 (SD−1).

Results: Our mouse efficacy testing showed that oral administration of SD−1 at 0.8−1.6mg/kg B.W. caused male infertility due to sperm deformation and lack of progressive motility in ~4 weeks. Continuous SD−1 treatment maintained the male infertility indefinitely, and male fertility was regained in ~4 weeks after the cessation of SD−1 treatment. During SD−1 treatment, no significant decrease in either testis weight or sperm counts was observed despite that close to 100% of epididymal sperm were deformed and display minimal or no progressive motility. Moreover, no any discernable side effects were observed and pups fathered by males recovered from SD−1−induced infertility were developmentally normal, healthy and fertile.
Conclusion: Our data suggest that SD−1 is a very promising oral male contraceptive agent.

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HIDE AND SEEK WITH SPERMS:MICROTESE AN OPTION IN NON OBSTRUCTIVE AZOOSPERMIA

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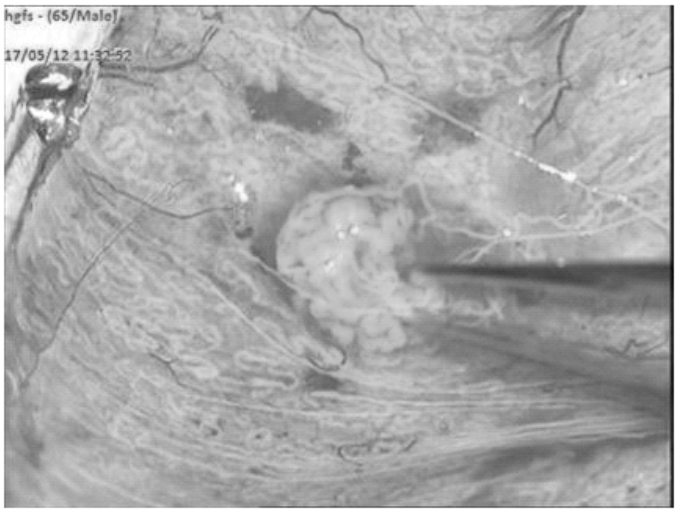
(Presented By: Dharmaraj Palanisamy, DNB (UROLOGY))

Introduction and Objective: Nonobstructive azoospermia(NOA) is an unfavourable prognostic condition for male infertility since spermatogenesis is disrupted at various levels. Sperm retrieval (SR) coupled with intracytoplasmic sperm injection (ICSI) is the only option for men with NOA seeking infertility treatment. Among the SR techniques, microdissection testicular sperm extraction (micro−TESE) has been applied with encouraging results. We present micro−TESE experiwnce in 150 patients with NOA and poor prognosis for SR.
Methods: Case series of men (n− 150) with NOA treated in a tertiary health care center Assisted reproductive technology (ART) facility was setup to perform SR using microsurgery. 150 men with NOA and prior failed retrievals or unfavourable histological results underwnt micro−TESE while their female partners underwent ovarian stimulation with oocyte retrieval(OCR). Micro− TESE was performed a day prior to OCR and testicular sperms were used for sperm injection. We assesed the retrieval rate and ICSI outcome. Outcomes of SR and ICSI were analysed descriptively. Mann Whitney and Fisher exact test were used to compare characteristics of men with successful and failed SR.
Results: The success of M−TESE is 50% in retrieving sperms with no major complications. A clear microscopic distinction between enlarged and collapsed tubules was seen in 33% of cases and sperms were retrieved in all except few. Patient with successsful and failed retrieval does not differ with respect to baseline characteristics, use of medical therapy, teticular biopsy. Sperm injection resulted in normal fertilisation and embryo cleavage of 64% and 76%. A total 50 embryo transfers with an average of 1.5 embryos resulted in cumulative clinical pregnancy rate per ICSI cycle of 30% with implantation rate of 34%.
Conclusion: We are successful in integrating the M−TESE procedures to the IVF laboratory. Our experience with micro−TESE applied to most difficult case of azoospermia is reassuring.



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ABSORBABLE CYANOACRYLATE FOR USE IN MICROSURGICAL VASOVASOTOMY:A NOVEL METHOD TO REINFORCE THE ANASTOMOSIS

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(Presented By: Tariq S. Hakky, MS)

Introduction: The absorbable cyanoacrylate surgical sealant (Ethicon OMNEX Surgical Sealant; Closure Medical Corporation, Raleigh, NC) has been applied to vascular surgery to seal and strength the anastomsis. This sealant adheres to the tissue or synthetic material, creating a flexible seal that prevents leakage of fluid in the presence of air, tissue and blood in within 1–3 minutes. We applied this applied this sealant during microsurgical vasovasotomy to seal and strength the anastomosis, decrease operative times, decrease risk of leak from the anastomosis site.
Methods: After an IRB was obtained, we performed a simple vasovastomy on four patients who requested vasectomy reversal. Once the vassal ends were cut we used four 9−0 prolene sutures. These sutured were placed at the 12, 3, 6, and 9 o'clock positions. Once the sutures were tied down the sealant was applied to a dry surgical field. We then allowed it to set for 120 seconds prior to releasing the two vassal ends. Patients had scheduled semen analysis at 3 months and 6 months. The primary endpoint was decreasing operative times from traditional one or two layered microsurgical anastomosis. The secondary endpoint included positive semen analysis post reversal. Inclusion criteria included any man who had undergone a vasectomy within the last 10 years and requested reversal. Patients were excluded if they had any prior scrotal surgeries other than vasectomy, if they did not wish to participate in the study, if their vasectomy was performed more than 10 years ago and if the patient required vasoepididymostomy.
Results: Four patients underwent microsurgical vasovastomy the mean time from vasectomy was 6.3 years. Three patients have semen analysis demonstrating the presence of sperm at the 3−6month follow up period. The fourth patient was lost to follow up. Mean operative times of (10−14) two−layered and (6−8 suture) one layered microsurgical simple vasovastomy in our institution is 320 and 155 minutes. Single layer closure with the use of absorbable cyanoacrylate is 63 minutes (50−90 minutes).

Conclusions: The cyanoacrylate surgical sealant was found to be safe and effective in the setting of microsurgical vasovastomy. It was associated with significantly decreased operatives times and did not interfere with semen passage through the anastomosis.

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ABSENCE OF NHERF−2 IN EPIDIDYMIS INCREASES LUMINAL SIZE THROUGH DYSREGULATION OF V−ATPASE LOCALIZATION.

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(Presented By: Wayland Hsiao, MD)

Introduction: Research on epididymal cell cross talk has shown that this process depends on the maintenance of sperm quiescence; specifically, the production of an acidic epididymal luminal environment through the proton pump V−ATPase. One membrane channel associated with the maintenance of low pH has been CFTR and apical expression in principal cells has been suggested as a regulator of V−ATPase. CFTR function in the nephron is regulated by Na+/H+ exchanger regulatory factors, NHERF−1 and NHERF−2. We hypothesize that NHERF−1 and NHERF−2 are involved in apical localization of CFTR leading to apical localization of V−ATPase.
Methods: We examined the morphology of epididymal tubules in NHERF−2 KO mice and found that the dimensions from the body and tail of KO mice were significantly larger when compared to age matched, wild type mice. Immunohistochemistry demonstrated that NHERF−2 expression can be seen in proximal portion of body of control mice, while V−ATPase and NHERF−1 expression begin the body and increase distally. NHERF−2 KO mice have a reduction in apical V−ATPase expression despite an elevated expression of total protein.
Results: Results suggest a lack of NHERF−2 leads to dysregulation of V−ATPase expression through upstream alterations in luminal environment. The increased size of the NHERF−2 KO epididymis body and tail may reflect the storage of increased immotile sperm due to increased pH.
Conclusion: We believe that this identification of a unique regulator of V−ATPase localization can elucidate the physiological mechanism of sperm maturation, leading to a potential treatment for male infertility and a pharmacological target for male contraception.

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INCIDENCE OF MALE AND FEMALE STERILIZATION FOLLOWING A RECENT LIVE BIRTH:
ESTIMATES FROM THE PREGNANCY RISK ASSESSMENT MONITORING SYSTEM (PRAMS), 2007−2010

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(Presented By: Lee Warner, PhD)

Introduction and Objective: Although contraceptive use is recommended postpartum, little is known about use of non−reversible contraception during this period.

Methods: We analyzed data from women from 15 states and New York City who participated in the 2007−2010 Pregnancy Risk Assessment Monitoring System (PRAMS). PRAMS is an ongoing, population−based surveillance system of women surveyed 2−6 months following delivery of a live birth. Use of non−reversible contraception after a recent live birth was assessed. Among women using contraception following delivery, we used polytomous logistic regression to separately assess predictors of tubal ligation and partner vasectomy compared with reversible contraception.
Results: Among 48,519 women who recently delivered a live birth, 11.1% (95% CI: 10.6−11.5%) reported having a tubal ligation (ranging from 6.6% in Utah to 20.8% in Mississippi) while 3.4%(95% CI: 3.2−3.7%) reported their partner had a vasectomy (ranging from 1.2% in New York City to 4.9% in Missouri). The ratio of tubal ligation to vasectomy use significantly exceeded 1 in all reporting areas, ranging from 1.9 in Utah [tubal ligation: 6.6%; vasectomy: 3.5%] to 10.4 in Mississippi [tubal ligation: 20.8%; vasectomy: 2.0%]. Multivariable modeling revealed that, compared with reversible methods, vasectomy following recent live birth was associated with being married [aOR=2.1 (95% CI=1.4−3.1)], having >=1 prior birth [eg, 4th birth vs 1st, aOR=19.2(12.8−28.9)], increased maternal age [>35 vs 20−24, aOR=2.7(1.8−4.0)], and increased maternal/paternal education [>=high school vs <high school, aOR=1.3(1.0−1.8) and aOR=1.3 (1.0−1.7), respectively]. Tubal ligation was associated with having >=1 prior birth [aOR=33.0(24.8−44.0)] and increased maternal age [>35 vs 20−24, aOR=4.2(3.4−5.2)], but inversely associated with being married [aOR=0.8 (0.7−0.9)] and maternal/paternal education [aOR=0.7(0.6−0.8) and aOR=0.6(1.0−1.7), respectively]
Conclusions: Although use of female sterilization was more common than male sterilization following delivery of a live birth, one in four women using non−reversible contraception reported their partners had a vasectomy. PRAMS data suggest significant variation by state in use of female versus male sterilization as well differences in education and marital status for users of these methods.

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PIOGLITAZONE INCREASES CYCLIC GMP CONCENTRATIONS IN A RAT MODEL OF POST−PROSTATECTOMY ERECTILE DYSFUNCTION

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(Presented By: Louis Aliperti)

Introduction: Erectile dysfunction (ED) is a common complication of radical prostatectomy. Pioglitazone (PIO) is a thiazolinedione derivative used in the treatment of diabetes mellitus. Given its known vasculoprotective and antifibrotic properties, we evaluated pioglitazone in a rat nerve−crush model of ED.

Methods: 15 Sprague−Dawley rats were stratified into three groups: 1−sham, 2−nerve crush (NC), 3−PIO treatment. Sham rats underwent an abdominal incision. Groups 2 and 3 underwent bilateral cavernosal NC. All rats subsequently underwent oral gavage(sham and NC with phosphate buffered saline, PIO treatment with PIO 0.65 mg/kg). Following a 1−day washout period, all rats underwent cavernosal nerve stimulation at 7.5V. Intracavernosal pressure to arterial pressure (ICP/MAP) was assessed as a measure of erectile function. Corporal tissue was snap frozen and analyzed for cGMP by ELISA (Cayman Chemicals Inc.). Statistics were performed using Student's t−test, with $p<0.05$ as significant.
Results: A significant decrease in ICP/MAP was observed in NC rats compared to sham animals at all voltages. However, PIO−treated animals showed voltage−dependent increases in ICP/MAP values compared to NC controls of 0.62±0.05 vs 0.42±0.05, p=0.0229, respectively. Increases in cGMP concentration were observed in PIO treated rats compared to control animals. cGMP levels in sham were 35 ± 3.5; in NC 30.4 ± 3.1; in PIO 45 ± 13.9 pmol/g (p=0.22).
Conclusion: PIO administration improves erectile function in a post−prostatectomy ED model via a cGMP−dependent pathway.

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THE PENILE DOPPLER PARAMETERS AND CLINICAL RISK FACTORS IN MEN WITH ERECTION HARDNESS SCORE 3−4 AFTER INTRACAVERNOUS INJECTION

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(Presented By: Qiang Li)

Introduction and Objectives: The Erection Hardness Score (EHS) is a simple, valid, reliable instrument to measure erection outcome and the ability for penetration. The objective of this study was to determine the best penile Doppler (PD) parameters and clinical risk factors for predicting an EHS 3−4 after intracavernous injection.
Methods: Among a total of 462 patients who underwent a PD ultrasound after intracavanous injection between July 2008 and February 2013, 221 (48%) patients achieved EHS 0−2 and 241 (52%) patients achieved EHS 3−4. The PD parameters were compared between the two groups using the Student's t test and the distribution of erectile dysfunction (ED) risk factors was determined using Chi−square test. The odds ratios (OR) of EHS 3−4 associated with PD parameters or ED risk factors were determined using a multivariable logistic regression model.
Results: Compared to patients with EHS 0−2, patients with EHS 3−4 were more likely to be younger (54 years vs 59 years, $P<0.001$) and showed significantly larger artery diameter (0.8 mm vs 0.6 mm, $P<0.001$), higher peak systolic velocity (PSV) (45.5 cm/s vs 28.5 cm/s, $P<0.001$), and lower end diastolic velocity (EDV) (0.4 cm/s vs 1.6 cm/s, $P<0.001$). EHS 3−4 was significantly associated with the presence of Peyronie's disease (p=0.01), and the absence of hypertension (p=0.001) or prostate cancer (all treatment modalities) (p=0.007). Multivariable analysis showed artery diameter (OR=14, $p<0.001$) and PSV (OR=1.03, $p<0.001$), but not EDV or resistive index, were independently associated with EHS 3−4. Patients with a history of hypertension or prostate cancer were half as likely to have an EHS 3−4 compared to patients without a history of hypertension or prostate cancer. (OR=0.5, 95%CI 0.3−0.8 p=0.005; OR=0.5, 95%CI 0.3−0.9 p=0.03, respectively).
Conclusions: The artery diameter and PSV are the strongest predictors of EHS 3−4, and hypertension and prostate cancer negatively affects EHS after intracavernous injection. Penile Doppler continues to be an indispensable tool to evaluate men with ED.

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SEXUAL FUNCTION IN MALE PARTNERS OF WOMEN PARTICIPATING IN A SURROGATE MOTHERHOOD PROGRAM

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(Presented By: Ioannis Giakoumakis, MD)

Introduction: We evaluated the sexual function of male partners of women participating in a surrogate motherhood program (SMP).
Methods: The international index of erectile function−5 (IIEF−5) outcome was calculated in 18 healthy sperm donors (group A), in 13 male partners (group B) of women participating in an in vitro fertilization (IVF) program, and in 16 male partners (group C) of women participating in an SMP. There were no significant differences in the mean value of age and peripheral serum testosterone among groups A, B and C.
Results: The mean IIEF−5 score was significantly smaller (P smaller than 0.05; Wilcoxon test) in group C (equal to 15) than in group A (equal to 22) and in group B(equal to 20). In contrast there were no statistically significant differences (P larger than 0.05) in the mean IIEF−5 score between groups A and B.
Conclusion: The significantly lower values of IIEF−5 outcome in group C compared with groups A and B may be attributed to an enhanced stress that experience the couples that participate in a SMP. Male partners of women who participate in an SMP have the hope and a strong desire one day to father their own children. However an SMP is a 20 to 30 times more expensive than an IVF program. In addition the biological parents have to participate in a legal recourse to confirm that the pregnant surrogate mother will give the child to the biological mother immediately after delivery. This results in an additional amount of stress for the biological parents. Furthermore stressful discussions concerning the financial reimbursement of the surrogate mother are necessary between the biological parents and the surrogate mother.

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THE EFFECT OF ANTIOXIDANT TREATMENT ON SEMINAL VESICLES AND VAS DEFERENS FUNCTION IN THE DIABETES MELLITUS RAT MODEL.

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(Presented By: Panagiota Tsounapi, PhD)

ABSTRACTS

Introduction and Objectives: Diabetes Mellitus (DM) is one of the high growing diseases threatening the human health. The incidence of DM is increasing rapidly usually affecting neurological, endocrinological and reproductive functions. Previous studies reported that DM also affects the sexual function of humans, or animal models, and also cause ejaculatory disorders. In this study we investigated the effects of DM in the seminal vesicles (SV) and vas deferens (VD) functions by employing in vitro organ bath studies. We also investigated if DM−induced dysfunction of SV or VD can be reversed by antioxidant treatment.
Methods: Control group was consisted of 10 rats (Control). Diabetes was induced in 40 rats by a single dose of STZ (50 mg/kg) i.p. Diabetic rats were divided in: non−treated DM rats (20 rats; group DM), DM rats treated with edaravone 10 mg/kg i.p. daily (10 rats; group DM+E), and DM rats treated with taurine 500 mg/kg i.p. daily (10 rats; group DM+T). The treatment lasted four weeks. After the completion of the treatment both SVs and both VDs were collected from all animals. SVs and VDs functions were evaluated by in vitro organ bath studies. Contractions were induced by norepinephrine (NE) or carbachol (Crb) for SVs, and for VD contractions were induced by NE. The serum testosterone profiles were measured.
Results: The organ weights for both SVs and VDs were significantly lower in the DM group compared to the Control. Treatment with both edaravone and taurine significantly increased the SV weights compared to the DM group, while only taurine significantly increased the VD weights compared to DM group. The in vitro organ bath studies revealed a significant hypercontraction of the seminal vesicles as induced by NE or Crb in the DM group compared to the Control group. Treatment with taurine or edaravone did not significantly alter the NE−induced hypercontractions observed in the DM group, while the Crb−induced contractions were significantly normalized by both treatments with taurine or edaravone compared to DM group. The VD from DM group demonstrated significant hypercontractions compared with Control group. Both taurine and edaravone treatments significantly normalized this abnormality observed at the DM group. Testosterone levels were significantly lower in all diabetic animals compared to the Control.
Conclusion: Although antioxidant treatments did not manage to increase testosterone levels, they significantly corrected the SV and VD functions.

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ENDOTHELIAL−ERECTILE DYSFUNCTION AND CARDIOVASCULAR RISK FACTORS RELATIONSHIP

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(Presented By: Libor Zamecnik, MD, PhD)

Objective: Endothelial monolayer plays a crucial role in the vasodilatation and hemodynamic events that leads to a normal erection. Endothelial dysfunction have been well established as one of the risk factors for developing both cardiovascular disease and erectile dysfunction (ED). This might explain the association between ED and coronary artery disease (CAD), as many men diagnosed with ED are at risk for a possible subsequent atherosclerotic CV event. Our aim is to determine the proportion of men with the diagnosed of ED that suffer from endothelial dysfunction, and its association with the CVD risk factors.
Methods: We evaluated endothelial function on 50 consecutive men with the diagnosis of ED who presented to our clinic. Endothelial function was determined using ENDO−PAT 2000 (Itamar Medical, Israel) by measuring. Post−occlusive reactive hyperemia index (RHI) on peripheral arterial tonometry. Endothelial dysfunction was ruled out when RHI values are above 2.07, and diagnosed when RHI is below 1.67. In between these two values lies a gray area, which represents a zone for possible risk of developing future endothelial dysfunction. Descriptive statistical analysis was performed. The relation with CV risk factors were also evaluated.
Results: Median age was 52 years (range 32 − 82). 16 (32%) patient were confirmed to have endothelial dysfunction based on RHI, 16 pts (32 %) were in the "gray zone", and 18 pts (36 %) were in a normal RHI range. The Cohort overall CVD risk factors: hypertension 31 pts (62 %), diabetes mellitus 12 pts (24 %), dyslipidemia 35 pts (70 %), obesity 30 pts (60 %), smokers 43 %,low−HDL cholesterol in 14 pts (20 %), testosteron defficiency11 pts (22 %), and waist circumference >102cm was observed in 37 pts (74 %). Only 5 pts (10 %) did not exhibit any risk factors and they all fell in the normal RHI group. Statistical significant correlation was observed when the relationship between endothelial dysfunction patients and diabetes (p 0,4865), 2 or more comorbidities (p 0,00368), and level of triglycerides was observed (p 0,4917).
Conclusion: In our Cohort 68% of the patients with ED were diagnosed endothelial dysfunction or at risk of developing endothelial dysfunction. Endothelia dysfunction in ED patient is associated with CV risk factors. ENDO−PAT 2000 might be a useful tool to determining endothelial dysfunction in ED patient.
Study was supported by grant: PRVOUK – P25/LF1/2

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EVALUATION OF SPERM DNA DAMAGE AND ANEUPLOIDY IN MALE SURVIVORS OF PEDIATRIC HODGKIN'S LYMPHOMA

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(Presented By: Sergey Moskovtsev, MD, PhD)

Introduction: Hodgkin's lymphoma is common in adolescents/young adults and survival rates exceed 90% on contemporary multi−modality protocols. However survivors are at significant risk of impaired future fertility. Alkylating antineoplastic agents are considered the primary cause of gonadal dysfunction. The objective of this study was to assess the impact of chemotherapy on sperm quality, DNA damage and aneuploidy in survivors of pediatric Hodgkin's lymphoma.
Methods: This is a retrospective cross−sectional study of male Hodgkin's lymphoma survivors treated at a single pediatric institution from 1985−2007. Eligible males were recruited from survivors attending an aftercare clinic who were aged ≥18 years and > 3 years from completion of therapy. Study participants completed a questionnaire, underwent urological examination and an evaluation of sexual hormones and semen analysis. In consenting non−azospermic participants assessment of sperm DNA damage and sperm aneuploidy was performed. Cumulative doses of alkylator agents were expressed as tertriles and as cyclophosphamide equivalent doses.
Results: Of the 38/49 (76%) eligible male Hodgkins' lymphoma survivors contacted; 15 enrolled and completed the study. Age of participants ranged from 21−35 years (mean 26 years) with a median time to assessment of 12 years (range 6−20 years) from diagnosis. The majority (10/15; 67%) had stage I/II disease. All were treated on alkylator containing regimens. On semen analysis 47% (n=7) were normozoospermic, 20% (n=3) oligozoospermic and 33% (n=5) azoospermic. The mean cumulative alkylator score was lower in normospermic survivors (2.4 vs 3.3 and 3.4 for oligospermic and azoospermic respectively). Sperm DNA fragmentation index was normal (<15%) in the normozoospermic survivors (n=6) and borderline (16%) in the oligozoospermic survivor tested. Aneuploidy (chromosomes 13, 18,21, X/Y) was slightly elevated at 3.46% ± 0.97 in four normozoospermic participants and significantly higher at 11% in the survivor with severe oligozoospermia.
Conclusion: Infertility remains a concern for male Hodgkin's lymphoma survivors. Of those who retain spermatogenic capacity, there appears to be no long−term risk of increased sperm DNA damage, but the observed increase in the aneuploidy rates requires further evaluation in a larger cohort.

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UPDATES FROM THE CENTERS FOR DISEASE CONTROL AND PREVENTION REGARDING PROGRESS IN MALE REPRODUCTIVE HEALTH

Lee Warner, PhD and Hubert Vesper, PhD
CDC
(Presented By: Lee Warner, PhD)

Introduction: The Centers for Disease Control and Prevention (CDC) has a longstanding history of conducting scientific and programmatic activities with direct relevance to male reproductive health. Topics that have been covered across the nation's leading public health agency range from contraceptive use and effectiveness, infertility, STD and HIV prevention, testing and treatment, unintended pregnancy, and the standardization of hormonal measurements to the effects of various occupational, environmental and physical exposures on male reproductive health function. Several publicly available, population−based surveys conducted by CDC, including the National Survey of Family Growth (NSFG), National Health and Nutrition Examination Survey (NHANES), National Health Interview Survey (NHIS), National Vital Statistics System (NVSS), and Pregnancy Risk Assessment Monitoring System (PRAMS) can also be used to examined key aspects of male health. Highlights from these and other CDC surveys and surveillance systems will be discussed and progress on incorporating data elements regarding male reproductive health into these systems will be reviewed. The presenters will also discuss opportunities for collaboration with CDC and recent progress on new initiatives regarding the reproductive health of men.

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OPIOID−FREE ANALGESIA FOLLOWING ROBOT−ASSISTED LAPAROSCOPIC PROSTATECTOMY (RALP)

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(Presented By: Xiao Gu, MD, PhD)

Objective: Opioid analgesia employed for pain control following abdominal and pelvic surgery have potential adverse events and can delay return of normal bowel function. To minimize its use, we utilized scheduled intravenous (IV) acetaminophen and ketorolac for perioperative analgesia following RALP.
Methods: Prospectively collected data from hospital records of consecutive patients who underwent transperitoneal RALP using perioperative IV acetaminophen and ketorolac for pain control were reviewed. All procedures were performed under general endotracheal anesthesia utilizing a balanced technique. The balanced anesthetic was not standardized with the exception that all patients received acetaminophen 1000 mg IV over a 15 minute infusion and ketoralac 30 mg IV prior to extubation. All patients were extubated in the operating suite and transported to the post anesthesia care unit (PACU) with supplemental oxygen by facemask and pulse oximetry monitoring. Acetaminophen 1000 mg IV was administered q6 hours post−surgery, while ketorolac 30 mg IV was administered at q8 hour intervals. Patients were provided a clear liquid diet and ambulating the evening of surgery. Once passage of flatus and tolerating a regular diet were confirmed, patients were discharged home. The hospital records were reviewed to quantitate both parenteral and oral opioid consumption.
Results: 69 patients had a median age of 62 years and an American Society of Anesthesiologists (ASA) class of 3. Median operative time was 90 minutes and estimated blood loss was 75mL. Mean hospitalization and urethral catheter duration were 21.0 hours and 5.0 days, respectively. 22 (31.9%) patients received parenteral opioid medication in the PACU, but did not require opioid medication on the hospital floor; le 39 (56.5%) patients did not require administration of parenteral/oral opioid analgesia in the PACU/hospital floor. No immediate/delayed adverse events were noted.
Conclusions: Perioperative scheduled IV acetaminophen and ketorolac are effective for pain management following RALP. Use of this regimen has the potential to decrease the need for postoperative opioid analgesia for this procedure, thereby lowering the risk of opioid−associated adverse events.

ABSTRACTS

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CORRELATION OF IMMUNOBEAD AND IMMUNOSPHERES IMMUNOGLOBULIN G (IGG) TESTS ON DETECTING ANTI-SPERM ANTIBODY (ASA) ON SPERM

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(Presented By: Aniela Bollendorf, MT, HEW)

Introduction and Objective: Production of the direct immunobead test for detection of sperm laden with antisperm antibody in phasing out. One consideration is to perform the test with immunospheres. The question is how well do they correlate.
Methods: The new direct immunosphere test for IgG is performed by mixing live motile sperm with latex beads coated with antibodies that bind to human IgG antibodies. The beads are first washed with a medium containing 1−2% bovine serum albumen and can be stored up to 3 days at 4oC. Sperm is diluted to give a final concentration of 10x106/mL. Five microliters of sperm suspension is mixed with 5 microliters of anti−IgG beads. After 1−2 minutes 150 motile sperm are counted and the percentage of sperm having beads attached is determined.
Results: There were 29 samples that were split and the presence of ASA was measured by immunobead and immunosphere test. There were 11 immunobead specimens read as zero and all 11 were similarly read as zero with immunosphere. There were 14 specimens read as zero by immunosphere with 4 slightly discordant immunobead tests read as 3, 2, and 7%, respectively. There were 11 immunobead specimens read as 100% ASA with complete agreement with immunosphere in 3. 98−99% in 3, and the others showing 95%X2, 92% and 83%, and 64%. One immunosphere read as 100% and the corresponding immunobead was 97%. There were some larger discrepancies however. One sample was 87% by immunobead read as 31% immunosphere. Other samples showed 7 vs. 0, 48 vs. 42 and 97 vs. 87.
Conclusions: There appears to be a good correlation between measuring ASA by immunobead vs. the immunosphere. Some andrologists consider with immunobead ASA levels >50% and some consider >80% as clinically important. Using the 50% cut−off value for ASA there was only 1 male with positive ASA by immunobead but negative by immunosphere and only 2 if the 80% cut−off was used.

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COX−2 AND TLR−4 AS NEW PUTATIVE BIOMARKERS OF CHRONIC INFLAMMATION IN LEUKOCYTOSPERMIA

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(Presented By: Sharika Hagan)

Introduction and Objective: Leukocytospermia (LCS) is a common cause of male infertility. Most often it is due to prostatitis and genitourinary inflammation (GUI) manifested by increased number of white blood cells (WBC), inflammatory chemokines, and reactive oxygen species (ROS) in the seminal plasma leading to decreased sperm motility and functionality and high sperm DNA damage. Many times it is idiopathic and chronic. There is an urgent need to develop much sensitive biomarkers for effective treatment of LCS before it gets to chronic stage. The current study explores newer inflammatory biomarkers such as toll−like receptor−4 (TLR−4); cyclooxygenase−2 (COX−2); and oxidative stress regulating antioxidant transcription protein, NF−E2−related factor 2 (Nrf−2) that counteracts the effects of ROS.
Method: Semen samples (n=60) collected from infertile patients (25 from non−LCS and 35 from age−matched LCS) were evaluated for sperm counts; motility/progression; morphology; and total WBC count. A differential expression profile of 60 inflammatory cytokines was determined by a commercial human cytokine antibody array (Ray Biotech; C−Series). Newer markers (TLR−4, COX−2, and Nrf−2) were evaluated by quantitative immunofluorescence microscopy (IFM).
Results: Semen samples from LCS patients showed significant decrease in sperm motility ($p<0.045$), progression ($p<0.005$), morphology ($p<0.05$) along with significant increase in WBC levels ($p<0.001$) as compared to non−LCS patients. Cytokine arrays revealed up−regulation of several pro−inflammatory cytokines and chemokines (mainly GM−CSF, IFN−γ, IL−7, MCP−2) in semen of LCS patients. The IFM data showed significant 7−fold increase ($p<0.001$) in TLR−4 and 5−fold increase ($p<0.01$) in COX−2 expression, while Nrf−2 expression showed significant 10−fold decrease ($p<0.01$) in LCS samples compared to non−LCS samples. Interestingly, these biomarkers were highly expressed in the nuclei of sperm head and in tail segments but showed much lower expression in the mid−piece section of spermatozoa collected from LCS patients, when compared to non−LCS samples.
Conclusions: These unique findings suggest that both TLR−4 and COX−2 can serve as novel biomarkers of leukocytospermia during chronic inflammation. Also, their differential localization in spermatozoa especially during GUI needs further exploration to understand their diagnostic and physiological role in male infertility practice.

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SPERM PROCESSING BY SINGLE DENSITY GRADIENT CENTRIFUGATION SELECTS FOR NORMAL HEAD, MID-PIECE AND TAIL MORPHOLOGY.

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(Presented By: Amin Herati, MD)

ABSTRACTS

Introduction: Semen analysis remains the cornerstone in the evaluation of male infertility. Motility and morphology are important parameters of the semen analysis and critical indicators of semen quality and fertility potential. Semen processing techniques have made it possible to enrich a sample for motile sperm, however, data are lacking on the morphologic profiles of these sperm. The objective of our study is to compare the morphologic profiles of an unprocessed semen specimen (unprocessed) and a semen specimen centrifuged over a colloidal silica separating media (processed) and analyzed using computer−assisted semen analysis (CASA).

Methods: Semen was prospectively collected from 18 men after at least two days of abstinence. Raw sperm semen parameters are determined with morphologies by CASA (SCA® Microptic SL, Barcelona, Spain). A 0.5 cc aliquot of raw sperm was centrifuged at 300 g over a 0.5 cc of 90% colloidal silica separating media (ISolate®, Irvine Scientific, Santa Ana, CA, USA). Computer−assisted semen analysis was then performed to determine the morphologic profiles of the processed sperm. Paired t−tests were performed for all data with 2−tailed Wilcoxon signed rank t−test where appropriate for data with nonparametric distribution.

Results: The mean volume (±SEM) for the whole cohort was 2.9 ml (±1.3), and the median days of abstinence was 3 days (range, 2−5 days). Using Kruger's strict criteria for morphology, the mean percent of normal forms in the processed sperm was significantly higher than in the raw specimen (14.7±1.9 vs 11.5±1.4; p=0.05). Similarly, the percentage of sperm with normal midpiece morphology was higher in the processed specimen (71.0±3.5 vs 61.3 ±2.7, p <0.005). Sperm isolated with processing had a statistically significant lower percentage of macroheads, piriform shaped heads, abnormal midpiece size, abnormal midpiece insertion angles and short tails.

Conclusion: Processed sperm demonstrated a higher percentage of normal head, midpiece and tail morphology. Specifically, specimens after processing have less macroheads, piriform shaped heads, abnormal midpiece sizes, abnormal insertion angles and short tails than raw sperm. Our data suggest that processing enriches the specimen for normal morphology. Additional studies are needed to determine if the processed sperm morphology more accurately reflects sperm function than the current standard.

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THE EFFECT OF CIGARETTE SMOKING QUANTITY ON SEMINAL LEUKOCYTES AND SEMEN PARAMETERS

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(Presented By: Erik Yao, BSc)

Introduction: Pyospermia can result from inflammation, infection, trauma, or other genitourinary insults. Both pyospermia and cigarette smoking have been associated with impaired semen parameters. We sought to explore the association between smoking, pyospermia, and semen parameters.

Methods: Men presenting for a fertility evaluation from 2008−2012 reporting smoking cigarettes were identified in a prospectively collected database. Patients were divided based on quantity smoked: non−smokers, < 1 packs per day (PPD), 1 PPD, 2 PPD or more. Data were analyzed for lifestyle confounders (marijuana and alcohol use) and semen parameters.

Results: Of 2787 total men, 861 (30.9%) men reported that they were current smokers. Of smokers, 695 (80.7%) smoked < 1 PPD, 116 (13.5%) 1 PPD, and 50 (5.8%) 2 PPD or more. Men without semen analyses were excluded from analysis. Marijuana was more commonly used among heavy cigarette smokers: 86.9% among those using ≥ 2 PPD compared to 20.63% in those smoking 1 PPD, 24.4% in those smoking < 1 PPD. The level of alcohol consumption did not vary between smokers and non−smokers. The proportion of men with pyospermia (defined as > 1 x10^6/ml WBC in semen) was as follows: For non−smokers 2.70% (50/1855), for those using < 1 PPD: 4.71% (13/276: p=0.065 compared with non−smokers), those smoking 1 PPD: 13.89% (5/36: p<0.001 compared with non−smokers), and 2 PPD or more: 0 (0/11: p=0.581). Semen parameters within the groups are listed in Table 1. The total motile sperm count (TMC) was not different for smokers versus non−smokers, or within smoking groups: Non−smokers: 30.8 ± 54.9 x10^6, < 1 PPD (27.5 ± 55.9 x10^6), 1 PPD (16.2 ± 21.9 x10^6), 2 PPD or more: (54.8 ± 119.1 x10^6). The total sperm count (TSC) for non−smokers: 88.0 ± 131.2 x10^6 was significantly different than of men that smoked 1 PPD, 48.7 ± 98.3 x10^6 (p=0.018). Vitality was not different between groups.

Conclusions: Approximately ¼ of infertile men are smokers. As the quantity of smoking increases, so does the proportion of men with pyospermia. TSC was lower in the 1 PPD group as compared with non−smokers, however the TMC was not different among groups. This study is limited by a small sample size in the ≥ 2 PPD smokers category.

Table 1: Demographic data and semen parameters for infertile men, divided by amount of cigarettes smoked.

Smoking Quantity (PPD)	Sample Size	Age (years)	Volume (ml)	Concentration (10^6/ml)	Motility (%)	Morphology (%)	TSC (10^6)	TMC (10^6)	Vitality (%)	% of PBMs >1 x 10^6/ml
0	n=2202	37.1 ± 6.2	2.8 ± 1.6	34.5 ± 60.1	23.9 ± 14.5	13.6 ± 10.1	88.0 ± 131.2	30.8 ± 54.9	67.1 ± 15.3	2.70
<1	n=480	36.3 ± 6.4	3.2 ± 1.6	26.3 ± 43.9	23.4 ± 14.7	12.3 ± 9.7	67.5 ± 120.6	27.5 ± 55.9	66.1 ± 17.2	4.71
1	n=63	37.0 ± 6.7	2.5 ± 1.4	22.6 ± 44.8	23.2 ± 14.1	15.3 ± 9.4	48.7 ± 98.3*	16.2 ± 21.9	67.7 ± 12.5	13.89*
≥2	n=23	36.0 ± 5.8	3.3 ± 1.9	30.7 ± 70.7	27.0 ± 21.1	16.2 ± 13.2	84.7 ± 119.1	54.8 ± 119.1	67.2 ± 8.4	0.00

* = significant at the p<0.05 level

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LOCALIZATION OF SYNAPSIN I IN HUMAN SPERM CELLS

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(Presented By: Jennifer Venditti, PhD, MS, BS)

Introduction and Objectives: Cell to cell signaling is a widespread process within organisms, and this signaling must be carefully regulated by numerous proteins. Fertilization is a carefully orchestrated cascade of events that requires communication between both the sperm and oocyte. Certain proteins known to have functions in neurons and other types of secretory cells have recently been shown to be present in human sperm. One such group of proteins, the synapsins, has been very well characterized in neurons, but very little is known about synapsin function in other types of cells. The goal of this project was to investigate the localization and distribution of synapsin in human sperm cells using immunocytochemical and protein blotting techniques.

Methods: Human semen samples were washed to remove unwanted cellular debris and seminal plasma components. Sperm cells were fixed onto a microscope slide with methanol. For immunolocalization, slides were incubated with a blocking solution for a minimum of 60 minutes, followed by primary anti−synapsin I antibodies for 60 minutes at room temperature in a moisture box. Following PBS rinses, slides were incubated with a fluorophore−conjugated secondary antibody for 30 minutes at room temperature in a moisture box. Slides were again rinsed with PBS and mounted with fluorescent mounting medium and a No. 1 coverslip. Results from immunolocalization experiments were documented using epifluorescence microscopy. Protein extracts were prepared from human sperm, mouse brain, and mouse testis/epididymis samples. The protein extracts were evaluated for the presence of synapsin I using dot−blot and Western blot techniques.
Results: Immunolocalization experiments showed positive staining for synapsin I in human sperm cells. Synapsin I localized to the plasma membrane of the sperm head, and in some cases showed an enrichment in the equatorial segment. The presence of synapsin I in human sperm was confirmed by both dot−blot and Western blot techniques.
Conclusion: The localization of synapsin I to human sperm cells is a novel finding, as this group of proteins has been thought to primarily be present in neurons. This research will enhance our understanding of regulatory synaptic proteins, and the possible role of such proteins in fertilization and reproduction.

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ELEVATED NITRIC OXIDE LEVELS MEDIATE MOTILITY DEFECTS IN JAM−A AND PMCA4 NULLS: PMCA4 IS A NEGATIVE REGULATOR OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN MURINE SPERM

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(Presented By: Patricia Martin−DeLeon, PhD)

Introduction and Objective: Reduced sperm motility (asthenospermia, AS) is a primary cause of male infertility and a large proportion of the cases are idiopathic. In mice, AS leading to infertility results from deletion of the gene encoding the highly conserved Plasma Membrane Calcium ATPase 4 (PMCA4), the major Ca2+ efflux pump in sperm. We have reported AS in mice lacking Jam−A (Junctional Adhesion Molecule A), and have shown that it results from decreased activity of PMCA4. How the absence of PMCA4, or its reduced activity, leads to AS is unknown. Our goal was to determine the mechanism by which deletion of Pmca4 and Jam−A exert its effects on motility and ultimately leads to infertility.
Methods: Since nitric oxide (NO) plays a crucial role in motility and PMCA4 (in addition to its Ca2+ efflux role) is known to modulate nitric oxide (NO) signaling by negatively regulating NO production, via nitric oxide synthases (NOSs), we used immunofluorescence to localize PMCA4, endothelial (eNOS) and neuronal (nNOS) NOS in sperm. Co−immunoprecipitaion (Co−IP) was used to study the association of eNOS and PMCA4 in uncapacitated (UNCAP) and capacitated (CAP) sperm. Intracellular NOS activity and peroxynitrite (OONO−) levels were measured in UNCAP and CAP Jam−A and Pmca4 null sperm and compared to WT, using flow cytometry.
Results: eNOS and PMCA4 were co−localized on the proximal principal piece (PPP) and over acrosome. Co−IP assays revealed an association of PMCA4 and eNOS in CAP, but not UNCAP sperm. NOS activity was significantly elevated in CAP compared to UNCAP Jam−A and Pmca4 null sperm. Similarly the levels of OONO−, a highly reactive primary effector of NO were markedly increased in Pmca4 nulls.
Conclusions: Our results show that in sperm eNOS interacts with PMCA4 which negatively regulates it. They support our hypothesis that AS in Pmca4 and Jam−A null sperm results from elevated levels of NO and its reactive byproduct (OONO−) which causes lipid peroxidation of sperm membrane, a key factor in motility loss. Our data suggest that PMCA4 mutations may be involved in AS in humans and thus may be relevant for AS diagnosis

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JAM−A−CASK COMPLEX INTERACTS WITH CD9 TETRASPANIN AND AVB3 INTEGRIN TO MEDIATE CA2+ SIGNALING IN CAPACITATION AND THE ACROSOME REACTION IN MURINE SPERM

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(Presented By: Amal Al−Dossary, MS)

Introduction and Objectives: We have shown that Junctional adhesion molecule A (JAM−A) is essential for sperm motility and is involved in the maintenance of Ca2+ homeostasis via its PDZ−ligand interaction with calcium/calmodulin−dependent serine kinase (CASK). Our objectives were to determine if JAM−A−CASK complex in sperm is a component of a larger signaling complex seen in endothelial cells and if JAM−A becomes phosphorylated which is a requirement for its signaling activity.
Methods: Sperm were capacitated and induced to acrosome−react using Ca2+ionophore (A23187) and proteins extracted for co−immunoprecipitation assays. Immunofluorescence was used for colocalization assays.
Results: We identified tetraspanin CD9 as a novel interacting partner of JAM−A and CASK in sperm and have localized it on the midpiece, the proximal principal piece (PPP), and the over the acrosome where αvβ3 resides and where we have previously localized JAM−A. CASK, a membrane−associated scaffold protein, was shown to assemble a quaternary JAM−A−CASK−CD9−αvβ3 signaling−inactive complex in uncapacitated sperm. Upon capacitation a JAM−A−CASK binary complex dissociates from the quaternary complex and JAM−A is Ser285−phosphorylated. Ser285−phosphorylated JAM−A (pJAM−A) shows a dynamic spatial and temporal tail−to−head distribution in sperm. The level of pJAM−A decreased gradually from acrosome−reacted (AR) to capacitated (2−fold lower), to uncapacitated (2−fold lower) sperm. Further in AR sperm CASK, which in uncapacitated sperm is located in the PPP only, extends to the midpiece where its partners CD9, JAM−A,and αv reside.
Conclusion: The data suggest that JAM−A is phosphorylated by its interaction with CASK. As phosphorylated JAM−A is engaged in the activation of MAPK/ERK signaling and ERK signaling is involved in sperm function, our study identifies JAM−A, CASK, and CD9 as upstream components of the ERK pathway controlling motility, capacitation, and the acrosome reaction induced by Ca2+ ionophore.
Funded by NIH−RO3 HD073523 and NIH−5P20RR015588 to PAM−D.

ABSTRACTS

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SPERM MORPHOLOGY USING A NOVEL DICHOTOMOUS KEY ALGORITHM IMPROVES ANALYSIS STABILITY, REPRODUCIBILITY AND TEACHABILITY

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(Presented By: Anna−Marie Bort)

Introduction and Objectives: Most Strict morphology results show few normal sperm even in fertile men. In many centers, morphology no longer has predictive value for ART. Proficiency test data show variation exceeding acceptable and useful limits. The WHO 5th edition Semen Analysis Manual adopted the Strict criteria, but their reference limits are much higher than many labs upper values. Lack of a standardized method for applying sperm classification criteria results in many different subjective interpretations of normal and makes it difficult to teach. Our objective was to develop a rational, repeatable method to apply classification criteria that would be easy to learn.
Methods: Surveyed 99 international experts on classification of 155 sperm and analyzed entropy (agreement). Reviewed photos and definitions of normal, borderline and abnormal sperm from atlases and publications. Based on these and established methods of pathology and taxonomy classification, we developed a dichotomous key algorithm with 12 queries of sperm features. Borderline normal forms were classified as a separate category using definitions from Menkveld 1990. 782 archived semen smears were analyzed for % normal with the algorithm and compared to original subjective method values.
Results: Strict % normal median with the algorithm was 18%, compared to original 4% (WHO 5th reference medians for unscreened men 14%, fertile fathers 15%). The distribution of values of 782 smears was comparable to the WHO 5th reference ranges with less than 10% of the values in the 5th centile (<4% normal Strict morphology) and a median of 20%. Regression analysis of 180 samples showed excellent inter−observer correlation with a correlation coefficient of 0.9. The method was stable over 8 months of analysis with a trend line slope of 0. An unexpected benefit was a 50% reduction in analysis time. Because borderline sperm are classified independently, the algorithm can be used to determine % normal for Traditional and Strict morphology schemes simultaneously. The algorithm was taught at two American Society of Andrology Lab Workshop where participant surveys stated the method was easy to use and adopt.
Conclusions: This novel morphology algorithm provided repeatable and stable results, with values and distributions similar to WHO 5th reference ranges. The method reduces ambiguity, decreases analytic time and reduces subjectivity.
Funding: NIH Grant R43 HD044383−01 and NIH Life Study

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COMPARISON OF SPERM CONCENTRATION AND ANALYSIS TIME USING SPERMOCYTOMETER® AND IMPROVED NEUBAUER HEMACYTOMETER COUNTING CHAMBERS

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Introduction and Objective: Sperm counting is an essential component of semen analysis. The improved Neubauer hemacytometer is a counting chamber intended for use with blood, but often is used for semen analysis. Its 100 micron depth allows sperm to be found in multiple focal planes and it requires dilutions, both sources of significant error. It is reusable and must be cleaned, disinfected and examined for contaminants. The Spermocytometer® (Leja Netherlands) is a disposable counting chamber specifically designed for sperm counting without dilution. Its 20 micron depth keeps sperm in a single plane. Our objective is to compare sperm concentration obtained with Spermocytometer® and hemacytometer.
Methods: Sperm counts were obtained with both chambers from 40 discarded clinical semen samples and sperm quality control reagents from 11 different lots for a total of 95 data points. For the hemacytometer, concentration was determined from two dilutions analyzed in duplicate using the method described by WHO 4th Edition Manual on the Examination of Human Semen, 1992. For the Spermocytometer®, the samples were loaded directly into the chamber without diluting. Sperm were counted with the aid of a 10 X 10 eyepiece reticle grid with 100 squares total, 1mm x 1mm each (Klarmann Rulings, KR−406B). All sperm in one grid were counted, then the stage was moved to acquire five grid counts total. Concentration was calculated from the average number of sperm per square multiplied by the measured scaling factor of the reticle. The concentrations were compared and correlation coefficient computed.
Results: The differences between concentration from Spermocytometer® and hemacytometer were not significant. The correlation coefficient value was 0.99685.
Conclusion: The Spermocytometer® consistently produced the same answer as the hemacytometer for each sample across a wide range oßf sperm counts observed in routine lab practice. This suggests that the Segre−Silberberg effect reported for similar chambers was negligible and undetectable. The hemacytometer requires cleaning and disinfection (2 min), checking for contaminants (2 min), making and counting duplicate dilutions (3−4 min) and time for the specimen to settle (5 min). The Spermocytometer® requires a 2−3 minute wait before analyzing to allow time for the sperm to stop drifting. Using the Spermocytometer® saved approximately 8 to 10 minutes per analysis.
Funding: Fertility Solutions Inc.

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CONTENT OF TESTIS−SPECIFIC ISOFORM OF NA/K−ATPASE IS INCREASED AND RAFT− AND NON−RAFT POOLS OF THIS PROTEIN ACTIVATE SPECIFIC SIGNALING PATHWAYS DURING BOVINE SPERM CAPACITATION

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(Presented By: Gayathri Devi Rajamanickam)

Introduction: Capacitation comprises a series of structural and functional modifications in sperm, enabling fertilizing ability. In our previous studies, we demonstrated that incubation of bovine sperm with ouabain (a specific ligand for Na/K−ATPase) induced capacitation through a mechanism involving kinases and redistribution of ATP1A4, the testis−specific α4 isoform of Na/K−ATPase. The aim of this study was to investigate the mechanisms by which multiple signaling pathways are activated during ouabain−induced capacitation. Previous studies in somatic cells demonstrated that Na/K−ATPase interacts with lipid rafts during cell signaling. Furthermore, lipid rafts are present in bovine sperm. Therefore, we hypothesized that lipid rafts serve as a signaling hub for ATP1A4, facilitating activation of multiple signaling molecules during ouabain−induced sperm capacitation.
Methods: Raft and non−raft membrane fractions were prepared from fresh sperm collected from mature Holstein bulls using a non−detergent−based approach. Both fractions were characterised for their total content of protein and cholesterol, as well as morphology, fatty acid profile, and the presence of raft and non−raft markers.
Results: Under capacitating conditions (incubation of sperm with ouabain), content of ATP1A4 increased in the raft and non−raft fractions compared to uncapacitated sperm; these capacitation−associated increases in ATP1A4 were confirmed by immunoblotting (soluble sperm proteins) and flow cytometry−based approaches. That content of ATP1A4 remained similar in detergent−insoluble sperm protein fractions from capacitated and uncapacitated sperm excluded the translocation of this protein from subcellular compartments to the sperm membrane. Raft and non−raft membrane fractions differed in the relative content of phosphorylated signaling molecules. Whereas raft fractions predominantly contained phosphorylated caveolin−1 and Src, non−rafts fractions contained phosphorylated forms of ERK1/2 and EGFR.
Conclusion: In conclusion, we inferred that content of ATP1A4 increased during bovine sperm capacitation and that raft and non−raft pools of ATP1A4 may regulate distinct downstream signaling events leading to sperm capacitation. This study received funding from NSERC.

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ALOE VERA SP. IS AN ACCEPTABLE ALTERNATIVE TO EGG YOLK FOR PRESERVING CANINE SEMEN AT 5ºC − PRELIMINARY RESULTS

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(Presented By: Erika Oliveira, PhD)

Introduction: Diluents containing egg yolk are the most practical for preserving semen at low temperatures. However, due to the recent requirements for disease control and security with biological products, it has been suggested that animal products be eliminated from diluents used for semen conservation. Few studies have been performed on the effect of Aloe vera sp. in ram and goat semen. To date, in vitro evaluation of dog semen after cooling with use of Aloe vera sp. has not been studied.
Methods: Therefore, this study assessed the effect of 5 % (wt/vol) Aloe vera sp. in a Tris extender (T1) or in a coconut water powder extender (ACP−101) (T2) for preservation of dog semen at 5°C. The control group received Tris 20 % (vol/vol) egg yolk extender. For this, 3 ejaculates from 5 male dogs (1 ejaculate/week/dog) were used. Ejaculates were stored at 5°C. Kinetic parameters (curvilinear velocity – VCL; linear velocity – VSL; mean velocity – VAP, and linear coefficient – LIN), total motility (TM), and sperm membrane integrity (SMI) evaluated by fluorescent probes (CFDA/PI) were assessed at 0, 24, 48 and 72h after cooling.
Results: Before cooling, TM (%) for control, T1 and T2 was 67.9±19.9, 53.9±18.3 and 48.6±18.2, respectively, and control had the best average values from this time (P=0.019) to the end of the study. Treatments with Aloe vera sp. did not differ between each other through the study. Regarding kinetic parameters, after 72h of storage, it was observed that Control had the best values for VAP, when compared to the other treatments (P<0.05), and was similar to T1 for VCL and to T2 for VSL and LIN. These parameters are important for the progression of spermatozoa into cervical mucus and the penetration of zona pellucida of oocytes. Control also revealed best values (53.2±1%) for membrane integrity when compared to T1 (43.4±1%) and T2 (46.5%±1) during the 72h of storage (P=0.0001). To our knowledge, this is the first report regarding the use of Aloe vera sp. as a substitution for egg yolk in Tris and ACP−101® for preserving chilled dog semen.
Conclusion: According to our results, egg yolk still has the best characteristics for preserving the viability of chilled semen but the results observed with Aloe vera sp. are within the normal range for fertility in this species, we suggest that it can be used as a substitute for egg yolk for preserving dog semen for 72h at 5ºC.

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CALCIUM KINETIC IN BOVINE SPERMATOZOA ALTERED BY INHIBITION OF PHOSPHODIESTERASE

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(Presented By: Anthony Laroche, BScA Agr)

Introduction and Objective: Cyclic adenosine monophosphate (cAMP) is a second messenger having high physiological relevance in sperm functions such as motility, capacitation and acrosome reaction. Phosphodiesterases (PDE) are the enzymes involved in cyclic nucleotides degradation. So, we hypothesized that PDE are actively involved in sperm physiologic response. Eleven PDE families are found in mammals with different affinities for cyclic nucleotides and PDE inhibitors. However, still not much is known in term of regulation and contribution of PDE in bovine sperm physiological functions. The objective of this research project is to study the effect of a specific PDE10 inhibitor on capacitation in bovine sperm.
Methods: Freshly ejaculated bovine sperms were provided by the CIAQ (Centre d'insémination artificielle du Québec). The semen has been washed twice in Tyrodes HEPES−buffered medium (spTALP−H−PVA) and incubated (5% CO2, 37°C) for 5 hours in spTalp−BSA (6mg/ml) in presence of either a specific PDE10 inhibitor (papaverine) or a non−selective PDE inhibitor (IBMX, 3−Isobutyl−1−methylxanthine).

Results: The semen motility and progressive motility were determined by a Computer Assisted Semen Analysis (CASA) and no difference was observed between treatments. To assess calcium's (Ca) management into the sperm, the response to thapsigargin (TG), a non−competitive inhibitor of SERCA pumps (sarcoplasmic/endoplasmic reticulum Ca2+ATPase) was measured, causing Ca depletion of sperm's stores. The fluorescent probe INDO−1−AM was used and two different spectral intensities were measured depending its coupling to Ca. By using flow cytometer, it has been possible to measure several thousand of events of bull ejaculate's response to TG over a period of 7 minutes. To improve the analysis of TG response, we've developed a new approach of plotting Ca kinetic released in a sigmoid curve. Using this curve, it's possible to calculate different time response such as the time needed to reach the plateau of the sigmoid curve. Sperm incubated in control treatment has reached the plateau of the curve significantly latter (242±19 s) compare to papaverine treatment (174±11 s) and IBMX treatment (128±6 s).
Conclusion: In brief, the results show that PDE10's inhibition influence intracellular Ca and its kinetic released in bovine sperm. This new method of analyzing TG response opens on other avenues in the comprehension of sperm's physiology.
Funding: This project was made possible by the contributions of FQRNT, NSERC and L'Alliance Boviteq Inc.

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TWO SIMPLE METHODS THAT DECREASE VARIATION IN SEMEN ANALYSIS RESULTS: LESSONS FROM THE 2013 ANDROLOGY LABORATORY WORKSHOP (ALW) OF THE AMERICAN SOCIETY OF ANDROLOGY

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(Presented By: John R. Quigley, BS)

Introduction and Objectives: Semen analysis proficiency testing reveals variation among laboratories that would be unacceptable for many laboratory tests. Frequently used methods for determining count and motility have imprecision that reduces the value of the test result. The recent 2013 ALW on Semen Analysis Quality Control examined ways to improve precision. To test the effects that simple changes to sperm count and motility methods have on analytic variation.
Methods: Sperm Count: Photographs of a semen sample were created with a superimposed 10x10 counting grid. 18 participants counted the sperm in rows D and G and in all 10 rows. A high resolution video of donor semen was created for projection with a segment of untreated "live"sample and a segment of semen incubated at 56C for 5 minutes to immobilize sperm "immobilized". 15 participants analyzed the video using 3 methods: a) Estimation of % motile after viewing live segment; b) Natation: motile and non−motile sperm were counted while viewing live segment, % motile was calculated by dividing number motile by sum of motile and non−motile, multiplied by 100; c) Static: non−motile sperm were counted in live segment, then all sperm were counted in the immobilized segment, the % motile was calculated by subtracting number of non−motile sperm from total immobilized sperm to determine number of motile sperm, divided by the total immobilized sperm, multiplied by 100. To compare the variation between/among the methods, standard deviation (SD) coefficient of variation (CV) were calculated for each set of results.
Results: CV Count: Row D 37%, Row G 26%, average rows D, G 28%, 10 rows 13%. CV Motility: Estimation 21%, Natation 8%, Static 7%.
Conclusions: Counting all rows reduced sperm count CV by 2/3. Row selection influenced CV. Averaging 2 rows did not reduce CV. Objective methods for motility reduced CV by over 50%. Natation and Static results were not different, but most participants reported that Static was easier. The exercises demonstrated practical ways to reduce variation and improve precision.
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TERMINALLY DIFFERENTIATED, POST−PUBERTAL RAT SERTOLI CELLS RESUMED PROLIFERATION AFTER TRANSPLANTATION.

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(Presented By: Jannette Dufour, PhD)

Introduction and Objective: The current dogma that post−pubertal Sertoli cells (SC) are terminally differentiated and do not proliferate, has recently been challenged suggesting that mature nondividing SC can be reprogrammed to proliferate. We have observed proliferation of SC isolated from post−pubertal rat testes after transplantation. The objective of the current study was to confirm and quantify this observation.
Methods: In this study, nondividing SC isolated from 23−27 days−old post−pubertal rats were transplanted underneath the kidney capsule of NOD scid gamma (NSG) mice or Lewis rats that were injected with 5−bromo−2'−deoxyuridine (BrdU; to label proliferating cells) or saline daily. After 10 days graft−bearing kidneys, testis, spleen and intestine were collected and tissue sections were double immunostained for Wilms' Tumor 1 (WT1; a SC marker) and BrdU.
Results: WT1 positive SC within the grafts were positive for BrdU. Germ cell within the testis and cells within the spleen and intestine were also positive for BrdU, while SC within the testis were negative for BrdU. Quantification of BrdU labeled SC demonstrated that 7.4% and 9.2% of the total transplanted SC within the grafts were proliferating in NSG mice and Lewis rats, respectively. Interestingly, the percentage of BrdU positive SC was lower when SC were arranged in tubules compared to SC located randomly outside of the tubules.
Conclusion: These data indicate that nondividing SC resumed proliferation after transplantation, and further validates previous findings that SC are not terminally differentiated. Transplantation of SC could provide a useful model to study the regulation of SC proliferation in vivo.

ABSTRACTS

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RNA POLYMERASE II PAUSING IS CRITICAL FOR SPERMATOGENESIS AND MALE FERTILITY

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(Presented By: Prabhakara Reddi, PhD)

Introduction: Successful completion of spermatogenesis relies upon precise spatiotemporal expression of distinct subsets of differentiation markers within the seminiferous epithelium. Failure to express genes at the correct time leads to arrested spermatogenesis and male infertility. The transcriptional mechanisms regulating this process, however, are not understood. Our work has established that RNA Pol II pausing is critical for maintaining the timing of gene expression during spermatogenesis. Paused RNA Pol II at the promoter ensures precise and rapid onset of gene transcription. This mechanism is particularly relevant to spermatogenesis wherein synchronous transcription of cohorts of genes is critical for morphogenesis and differentiation. We have identified the TAR DNA binding protein of 43 kDA (TDP−43) as a key player in maintaining paused pol II at a target gene promoter in germ cells. TDP−43 is evolutionarily conserved and highly expressed in mouse and human testis. Here we report that TDP−43 is essential for spermatogenesis.

Methods: Conditional knockout of TDP−43 in germ cells or Sertoli cells led to maturation arrest and male infertility. Loss of TDP−43 in spermatogonia, induced by the Stra8−iCre deleter, led to failure of entry into meiosis. AmhCre−induced loss of TDP−43 in Sertoli cells caused qualitative changes in spermatogenesis. While it is well−known that germ cells express genes in a precise spatiotemporal pattern, work from several laboratories established that Sertoli cells also express genes in accordance with the stage of the seminiferous epithelium. We are testing the hypothesis that Pol II pausing is critical for maintaining the timing of gene expression in these cells and that loss of TDP−43 disrupts this in a subset of genes poised for transcription.

Results: We report that TDP−43 binds to NELF, a critical component of pol II pausing and predict that mechanistically, TDP−43 guides the sequence−specific recruitment of the pause machinery.

Conclusion: This work explores a transcriptional mechanism that likely regulates the expression of a third of all genes expressed in the seminiferous epithelium, as it does in the embryo. Study of TDP−43 is highly relevant clinically because abnormal TDP−43 function is linked to a number of neurodegenerative disorders. Our future work will determine if male infertility also falls under TDP−43 proteinopathies.

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

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(Presented By: Vasan Srini, DNB, Fellowship – Andrology)

Introduction and Objectives: To identify potential biomarkers for azoospermia by establishing the expression patterns of genes. Our objective is to derive a novel set of candidate biomarkers for non−obstructive azoospermia (NOA) and determine a threshold for the 'reliability' of the score, which might help in identification of the potential markers.

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. Genes differentially expressed with fold change >2 were identified (summarized in the table below):

Hybridization (conditions compared)	No. of genes	
	Up−regulated in condition 1	Down−regulated in condition 1
NOA vs. OA	541	557
NOA vs. Normal	1530	2093
OA vs. Normal	433	698

A new scoring system was followed which 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, 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. The approach involved biocuration, development of a database, and deriving a consensus expression pattern across 'comparable' multiple studies for each gene. The new database and associated software serve as a 'gene expression prediction platform' which performed better than any other system in providing straight forward expression information for randomly selected genes.

Conclusions: A) The gene expression platform for mammalian testis with silico analysis provides highly reliable information with higher reliability, as per the database, were repeated frequently in the experimental data set for similar conditions. B) The analysis of the experimental results also indicated a threshold level for the reliability of the score. C) New sets of potential biomarkers identified are very promising as they contain many novel genes which could be useful for basic research.

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THE HISTONE H3 DEMETHYLASE, KDM1A IS ESSENTIAL FOR THE DIFFERENTIATION OF SPERMATOGONIA AND THE SURVIVAL OF SPERMATOGONIAL STEM CELLS.

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Introduction: Spermatogenesis is a highly complex cell differentiation process fueled by spermatogonial stem cells (SSCs). The progression from a spermatogonial stem cell to a differentiating cell involves gene expression changes that are under epigenetic control. Epigenetic mechanisms governing gene expression involve histones and their modifiers which add and remove permissive or repressive marks from histone tails. The histone demethylase KDM1A removes gene−activating methylation on histone H3 at lysine 4 (K4). KDM1A can be associated with other histone modifiers such as the histone deacetylase 1 (HDAC1), which removes gene activating H3 acetylation, forming a protein complex that will induce the silencing of the chromatin. We had previously observed that KDM1A was present in SSCs, hence we hypothesized that this protein serves in the epigenetic regulation of SSCs biology.

Methods: To determine the function of KDM1A in SSCs we generated mice with a conditional knockout of Kdm1a (cKO) specifically in spermatogonia.
Results: Analysis of the cKO revealed that KDM1A is essential for spermatogenesis, as adult cKO males were sterile and lacked germ cells. Testes were collected from cKOs at post−natal days (PND) corresponding to the appearance of spermatogonia (PND6) and meiotic cells (PND10). At PND6, spermatogonia were still present in the cKOs, however, at PND10 very few cells with an abnormal morphology were observed in place of preleptotene spermatocytes. Moreover from PND10 to 21 the number of spermatogonia in the cKO testes decreased dramatically with no germ cell remaining at PND21. To understand what mechanisms were behind the disappearance of spermatogonia and the almost complete absence of meiotic entry, we analyzed the global epigenetic profile of germ cells in the cKO. At PND6, the cKO spermatogonia presented higher levels of H3K4 di−methylation and H3 acetylation as determined by immunofluorescence. We then used RNA−sequencing to examine how KDM1A loss alters the gene expression profile of isolated SSCs and the analysis of this data is in progress.
Conclusion: These results suggest that without KDM1A the epigenome of the spermatogonia is altered and indicate that KDM1A is a master epigenetic regulator of SSCs required for SSCs survival and spermatogonia differentiation.

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SERTOLI CELLS ENFORCE PERITUBULAR MYOID CELL FATE AND ARE ESSENTIAL FOR DIFFERENTIATION, PROLIFERATION AND RETENTION OF ADULT LEYDIG CELLS

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(Presented By: Lee Smith, BSc, PhD)

Introduction: The ageing of western societies, and the associated increase in obesity, brings with it an increase in prevalence of disorders such as cardiovascular disease, diabetes, loss of bone density, muscle strength, libido and erectile dysfunction, which are associated with reduced androgen levels in men. As Leydig cells (LCs) are the source of androgens in men, establishing the mechanisms which control LC development and function is crucial to our understanding of ageing and male health. Sertoli cells (SC) regulate testicular fate in the differentiating gonad and are the main regulators of spermatogenesis in the adult testis; however, their role during the intervening period of testis development, and in particular during adult (A)LC differentiation and function remains largely unknown.
Methods: To determine whether SCs are involved in LC development two transgenic mouse models were generated which allowed controlled, cell−specific ablation of SCs in pre− and postnatal life.
Results: Results show that the SCs are required: (i) to enforce a myoid fate on peritubular cells (PTMC) in prepubertal life (ii) to maintain the ALC progenitor cell population in the postnatal testis (iii) for development of normal ALC numbers and (iv) for retention of normal ALC numbers in adulthood. Furthermore, our data shows that fetal LCs function independently from SC, germ cell or PTMC support in the prepubertal testis.
Conclusion: Together these findings describe a new paradigm, which encompasses SC−mediated control of ALC development and function and has significant implications for our understanding of both male reproductive disorders, and wider androgen−related conditions affecting male health.

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E2A AND HEB REGULATE SERTOLI CELL FUNCTION AND FERTILITY IN THE MOUSE

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Introduction and Objective: Spermatogenesis requires the support of Sertoli cells, which are the only somatic cell population in direct contact with developing germ cells. The Sertoli cell lineage is specified in the embryonic gonad and the population expands in number until early postnatal life in most mammalian species. In the testis of adult animals, Sertoli cell number is stable and the ratio of germ cells per Sertoli cell is fixed. Although it is well established that Sertoli cell functions and absolute number are crucial for spermatogenesis, the molecular mechanisms governing their proliferation and maturation remains unclear. E proteins (E2A, HEB and E2−2) are basic Helix−loop−helix (bHLH) factors that have important roles in cell differentiation and proliferation. Results of previous studies revealed that Sertoli cells express E2A and HEB; however, the functional role of these factors is unknown. The overall aim of this study was to determine whether E2A and HEB play an important role in function of Sertoli cells.
Methods: To achieve this, conditional knockout mouse models were generated using mice bearing E2A/HEB floxed alleles and a Sertoli cell specific Amh−Cre transgene. Neither testis weight nor fertility was altered in mice with single inactivation of E2A or HEB compared to littermate controls. However, double deletion of E2A and HEB resulted in a sub−fertility phenotype.
Results: At postnatal week 8, testis weight of E2A and HEB double knockout animals was significantly reduced compared to littermate controls and epididymal sperm count was decreased by more than 50%. Moreover, significant reductions in both Sertoli cell and spermatogonial numbers were found in the double knockout animals, which was likely the underlying cause of reduced sperm output and the sub−fertile phenotype. Lastly, examination of testes at postnatal weeks 3 to 4 revealed a significant reduction in testis weight and delayed emergence of elongate spermatids for the double knockout males. Further assessment of testis weight of control, single knockout and double knockout animals suggested that E protein dosage not identity is the important factor.
Conclusion: Collectively, these findings indicate that normal maturation of the Sertoli cell population during postnatal development is influenced by the transcription factors E2A and HEB.
This research was supported by grant HD061665 awarded to J.M.O. from the National Institutes of Health.

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REGULATION OF THE PROLIFERATION AND DIFFERENTIATION OF ADULT LEYDIG STEM CELLS

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ABSTRACTS

Introduction: New Leydig cells appear in the adult rat testis after the pre−existing adult Leydig cells are eliminated with ethane dimethanesulfonate (EDS). PDGFRα+ cells were purified from the testes of adult Brown Norway rats after the animals received EDS. Depending upon culture conditions, these cells proliferated indefinitely or differentiated and produced testosterone, suggesting that the cells might be stem cells. In a second study, seminiferous tubules were isolated from the interstitium of Leydig cell−depleted testes. Culture of the tubules for one week resulted in a peak of cell division on the surface of the tubules, and then a return to basal division levels by week 2. With culture from weeks 2−4, 3βHSD+ cells appeared on the surface of the tubules, and testosterone was detected in the culture medium. These results suggest that there are stem cells on the surfaces of the tubules that divide and then cease dividing, and that the products of the divisions then give rise to the newly formed adult Leydig cells.
Methods: To begin to identify how Leydig stem cells are regulated, we screened 35 factors or their signaling molecule modulators for their effects on the division or differentiation of the stem cells, using the in vitro tubular culture system.
Results: Desert Hedgehog (DHH), PDGF−BB, FGF−2, activin, PDGF−AA, IL−1β, TGF−α and IGF−1 had stimulatory effects on cell proliferation. DHH, PDGF−AA, and inhibin had positive effects on cell differentiation. Wnt signaling inhibited cell differentiation while TGF−β inhibited both cell division and differentiation. Intriguingly, although both PDGF−AA and –BB had stimulatory effects on cell proliferation, they had completely opposite effects on the differentiation of the cells. PDGF−AA induced the cells to enter the Leydig lineage while PDGF−BB blocked the process. Interestingly, PDGF−BB may induce the cells to enter the myoid cell lineage.
Conclusion: These results suggest that Leydig stem cells may in fact be multi−potent cells, serving as the common stem cells of both Leydig and myoid cells. The use of the seminiferous tubule culture system has promise to be a good tool to examine Leydig stem cell niche and their functions despite the complexity of the tissue.
This work was supported by NIH grant R37 AG021092 from the National Institute on Aging.

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IMPORTIN PROTEINS IN SPERMATOGENESIS AND SPERM

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(Presented By: Kate Loveland, PhD)

Introduction and Objective: Importin family proteins were initially identified by their classical nuclear import functions in which they ferry cargo containing a nuclear localization signal (NLS). The importin α proteins are adaptors that link a cargo protein to the importin β1 for transit through the nuclear pore into the nucleus. Importins are essential for cell viability and development, including gametogenesis (Miyamoto et al, 2012, 2013 BBA), and we documented the differential synthesis of many importin proteins throughout spermatogenesis in rodent and human testes (e.g. Whiley et al, 2012 Int J Androl). To discover functions of individual importins, we undertook to (1) measure their stoichiometry during successive stages of spermatogenesis, (2) identify specific cargoes for individual importin α proteins, and (3) determine the cellular distribution of importin proteins in mature spermatozoa.
Methods: Relative amounts of importins α2, α3, α4 and β1 were measured in spermatocytes and round spermatids isolated (n=3) from adult Sprague Dawley rats by elutriation and Percoll gradients to >90% purity. Western analysis of cell lysates run alongside known amounts of recombinant importin proteins was used to estimate importin levels.
Results: We calculated that importin α2 levels were 2−fold higher in spermatocytes than in spermatids, whilst importins α4 and β1 levels did not differ. Next, to find binding proteins for individual importins in rat spermatocyte and spermatid lysates, we used recombinant importin α2 and α4 proteins for pull−down experiments followed by a comprehensive proteomics analysis. Amongst the 100 candidates, only 42 had a strong classical NLS, while 8 nuclear proteins had none, indicating importin αs bind to non−canonical sequences in many proteins. In addition, these new and our previous data (Miyamoto et al 2013) reveal importin binding partners that are not nuclear proteins, but are instead typically found in mitochondria or vesicle−associated. We next performed indirect immunofluorescence to localize importin proteins in formalin−fixed cauda epididymal sperm from C57BL6 mice. Antibodies to IMPα2, IMPα3, IMPα4 and IMPβ1 each bound distinct functional regions in mature spermatozoa.
Conclusion: Individual importin protein levels are tightly regulated for distinct roles during spermatogenesis. We propose they deliver cargo proteins to non−nuclear compartments during assembly of mature spermatozoa.

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MITOCHONDRIAL METABOLIC ACTIVITY ASSISTS WITH REGULATION OF STEROID PRODUCTION IN MA−10 MOUSE LEYDIG CELLS

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(Presented By: Malena Rone, PhD)

Introduction: Mitochondria are home to many cellular processes, including oxidative phosphorylation, fatty acid metabolism, and in steroid synthesizing cells, cholesterol import and metabolism to pregnenolone. The formation of macromolecular protein complexes aids in the regulation and efficiency of these mitochondrial functions, though due to their dynamic nature are hard to identify.
Methods: To overcome this problem we utilized Blue−Native polyacrylamide gel electrophoresis (BN−PAGE) coupled to mass spectrometry on isolated mitochondria from control and hormonally stimulated mouse MA−10 Leydig cells.

Results: The data obtained identified the presence of a number of qualitatively similar mitochondrial protein machineries, under control and hCG−stimulated conditions. In addition, quantitative differences were observed in mitochondrial complex formation after hormone stimulation as compared to control cells. A prominent decrease of mitochondrial proteins involved in fatty acid import into the mitochondria was observed. From these results we implied that mitochondrial β−oxidation is not essential for steroidogenesis. To confirm this we inhibited fatty acid import utilizing the carnitine palmitoyltransferase Ia (CPT1a) inhibitor etoxomir, resulting in an increase in steroid production after 24 hour incubation of the cells with the drug. Moreover, etoxomir induced a decrease in oxygen consumption with an increase in extracellular acidification, confirming the inhibition of β−oxidation. A shift towards glycolysis with no observed lost ATP production was also observed.
Conclusion: These results suggest that changes in the metabolic profile of the mitochondria in steroidogenic cells can function as a potential regulator in cholesterol import and steroid production. We propose that upon hormonal stimulation, the mitochondria efficiently import cholesterol at the expense of other lipids necessary for energy production resulting in their specialization for steroid biosynthesis.

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THE ANCIENT AND EVOLUTIONARILY CONSERVED REGULATORS OF PROTEIN PHOSPHATASE PP1, PPP1R2, PPP1R7, AND PPP1R11, ARE EXPRESSED AS TESTIS−SPECIFIC ISOFORMS DURING SPERMIOGENESIS.

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(Presented By: Nilam Sinha)

Introduction and Objective: Two of the four Ser/Thr phosphatase type 1 (PP1) isoforms, PP1γ1 and PP1γ2, are alternate spliced products derived from one gene, Ppp1cc. Their amino acid sequences are identical except at the extreme C−termini. PP1γ1 is ubiquitous whereas PP1γ2 is highly abundant in testis. PP1γ2 isoform is present only in mammals. Knock out of Ppp1cc, which eliminates both PP1γ1 and PP1γ2, results in male infertility. Expression of PP1γ1 in testis, using transgenic approaches, is not as effective as PP1γ2 in restoring male fertility in Ppp1cc null mice. Thus PP1γ2 appears to have an isoform specific role in supporting normal sperm motility and male fertility. Three PP1regulators, PPP1R2, PPP1R7, and PPP1R11 have been proposed to be present or identified in sperm. Based on their roles in regulating PP1 in somatic cells they are suggested to bind to and regulate sperm PP1γ2. The purpose of this study was to characterize sperm PPP1R2, which was first described using indirect biochemical approaches.
Methods: We were able to demonstrate by western blot and mass spectroscopy that PPP1R2 is present in spermatozoa.
Results: We discovered that an alternatively spliced message for PPP1R2 coding for a unique isoform that is abundant in testis. Amino acid sequencing identified the unique C−terminus of this PPP1R2 isoform in testis extracts. The message for this PPP1R2 isoform is present at high levels during spermiogenesis and in adult testis. Surprisingly, we also found that the other two PP1 inhibitors, PPP1R7 and PPP1R11, are expressed as testis−specific isoforms. The temporal patterns of expression of these two proteins also parallel that of PPP1R2 and PP1γ2 in testis. Testis PPP1R7 has a unique C−terminus due to alternate splicing, while testis PPP1R11 has a unique N−terminus due to an alternate transcription start site.
Conclusion: High levels of the three ubiquitous inhibitors expressed as testis−specific isoforms suggest involvement of these proteins in the isoform specific role of PP1γ2 in supporting normal sperm function and male fertility in mammals.
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NON−STEROIDAL LIGANDS OF THE CHOLESTEROL RECOGNITION AMINO ACID CONSENSUS (CRAC) MOTIF OF THE 18−KDA TRANSLOCATOR (TSPO) PROTEIN AND THEIR EFFECTS ON STEROID HORMONE BIOSYNTHESIS

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Introduction: Steroid hormone biosynthesis by mammalian gonads and adrenals is dependent on translocation of cholesterol across the double membranes of the mitochondria and delivery to the cytochrome P450 enzyme CYP11A1, where it is metabolized to pregnenolone, the metabolic precursor of all steroids. The 18−kDa translocator protein (TSPO), a high−affinity drug−binding integral membrane protein, located in the outer mitochondrial membrane has been implicated in this cholesterol delivery process. In addition to its drug−binding ability, TSPO is also a high−affinity cholesterol−binding protein, through a conserved Cholesterol Recognition Amino Acid Consensus (CRAC) motif located at its C−terminus. To better understand the possible roles TSPO and its CRAC motif may play in steroidogenesis, we have previously identified and validated a novel CRAC motif ligand, 5−androsten−3β,17,19−triol, which was able to inhibit hormone− and drug−mediated steroidogenesis. However, a non−steroidal ligand targeting TSPO's CRAC motif would be of interest both in increasing our molecular understanding of this protein motif and as a lead compound in the development of novel drugs for the treatment of diseases of steroid excess.

Methods: In this study, we computationally constructed a pharmacophore model of TSPO and its CRAC motif and utilized structure−based virtual screening identify CRAC−binding structures from approximately 11 million small molecular structures available from structural databases. The biological activity of the top−scoring identified molecules was subsequently tested in the TSPO−rich, hormone−responsive MA−10 mouse tumor Leydig cell line and constitutively steroidogenic R2C rat tumor Leydig cell line.
Results: A series of compounds was identified capable of inhibiting with nanomolar potencies steroid production in both of these cells. This inhibition was localized to the delivery of cholesterol to CYP11A1 in the mitochondrial matrix, as the cells retained the ability to synthesize steroids when supplied with 22R−hydroxycholesterol, a water−soluble cholesterol analog which bypasses the mitochondrial cholesterol−transfer step.
Conclusion: These results identify a novel family of non−steroidal compounds targeting TSPO's CRAC domain and potently inhibiting steroidogenesis, a family which may serve as useful tools in the study of TSPO function and steroidogenesis, as well as prove effective lead compounds for the development of drug treatments for maladies of steroid imbalance.

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HUSP26 EXPRESSION AND RELATIONSHIP TO ANDROGEN RECEPTOR IN NORMAL HUMAN TESTIS

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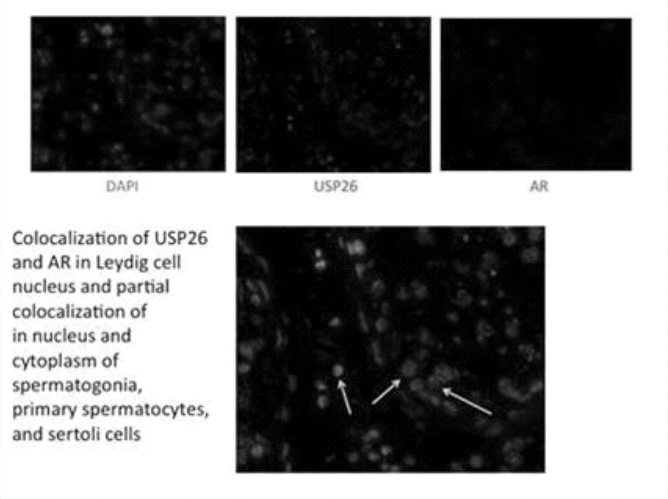
Introduction: Human ubiquitin specific protease 26 (hUSP26), an X−linked gene, is associated with male infertility and low testosterone production. hUSP26 has been recognized as a regulator of androgen receptor (AR) hormone−induced action involved in spermatogenesis and steroid production in in vitro studies. The goal of this study was to determine cellular localization of hUSP26 expression in normal human testis and its relationship to AR expression.
Methods: 3 patients with obstructive azoospermia at our institution had frozen testicular specimens available for measurement of hUSP26 and AR mRNA levels using multiplex qRT−PCR with LightCycler 480 (Roche). TATA−binding protein (TBP) was utilized for relative quantification and expression ratios were corrected with standard curves. Immunofluorescence colocalization studies were performed with paraffin−embedded and frozen tissues using primary and secondary antibodies to detect hUSP26 and AR protein expression.
Results: hUSP26 mRNA and AR mRNA is expressed in normal human testis. In normal human testis, USP26 and AR were colocalized in the Leydig cell nucleus with less in Leydig cell cytoplasm, spermatogonia, primary spermatocytes, and Sertoli cells (Figure).
Conclusions: USP26 mRNA expression and AR mRNA expression is present in human testis. USP26 colocalization with AR in Leydig and Sertoli cells, and early cells of spermatogenesis demonstrates possible interaction between these proteins in normal testis. The mechanism and implications of USP26/AR interaction in testis requires further study.



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ROLE OF ATAD3 IN THE HORMONE− INDUCED ER−MITOCHONDRIAL ORGANIZATION IN HORMONE−INDUCED LEYDIG CELL STEROIDOGENESIS

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(Presented By: Leeyah Issop, PhD)

Introduction: Leydig cell steroid formation is a multi−step process initiated in mitochondria, using cholesterol coming from intracellular stores, and finalized in the endoplasmic reticulum (ER). Cholesterol transfer from outer mitochondrial membrane (OMM) to CYP11A1 in the inner MM (IMM) is the rate−limiting step of this process and is dependent on the organization of the contact site formation. Studies on the characterization of the different proteins involved in this process, demonstrated a crucial role of the AAA+ATPase ATAD3 both in the regulation of cholesterol channeling and the integrity of contact site formation. ATAD3 is anchored in the IMM and enriched at OMM−IMM contact sites. The long isoform of ATAD3 possess an N terminus domain with 50 amino acids able to drive the insertion of the protein back into OMM. It is unclear however, whether this domain is involved in the complex making bridges between mitochondria and other cellular organelles, such ER. We hypothesized that the physical association between mitochondria and ER, named mitochondria−associated membranes (MAMs), can potentially regulate hormone−stimulated steroidogenesis.
Methods: Using the MA−10 mouse tumor Leydig cell line as a model and electron and confocal microscopy, we observed a significant increase of MAM formation upon hGC stimulation. MAMs were isolated and characterized with different specific markers such as ACSL4 and calnexin.

Results: Interestingly, we observed an enrichment of the long isoform in MAMs. Silencing ATAD3 resulted in reduced ability to form pregnenolone and progesterone in response to hCG treatment with no effect on 22−R hydroxycholesterol treatment, confirming the role of ATAD3 at the level of cholesterol delivery into mitochondria. Since progesterone is made mainly in the ER, and a profound modification of the mitochondrial inner structure was observed, we suggest that ATAD3 functions not only as a bridge between OMM−IMM but also might be involved in the organization of MAMs. MAMs could allow the transfer of the substrate cholesterol into mitochondria and steroidogenic pathway intermediates out of mitochondria. Deletion of the anchoring ATAD3 N−terminus blocked the hormone−induced steroid formation further supporting this role of ATAD3 in MAM formation.
Conclusion: Taken together, these results suggest a role of ATAD3 as a scaffold protein in the regulation of ER−mitochondria communications in Leydig cells, crucial for the optimal hormone−stimulated steroid formation.

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EFFECTS OF SILDENAFIL ON RAT SPERM DNA INTEGRITY

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(Presented By: Evlalia Vlachopoulou, BS, PhD)

Objectives: We evaluated the effects of sildenafil on rat sperm DNA integrity.
Methods: Group A included male Wistar rats (n=8, 8−week old) and served as a control group. Group B included male Wistar rats (n=8, 8−week old) that received daily an oral suspension containing 10 mg/kg of sildenafil for seven weeks. At the age of 15−week−old all rats were killed. Epididymal sperm content (ESC), the epididymal sperm motility (ESM;%), and the % epididymal caudal sperm with fully condensed chromatin (%SCC) was evaluated (Asian J Androl 2011,13:69).
Results: There were no significant differences in ESC or ESM between groups B and A (P larger than 0.05, Wilcoxon test). In contrast mean value of the %SCC was significantly larger in group A than in B (P smaller than 0.05).
Conclusions: The detrimental effect of sildenafil on sperm DNA integrity in the rat model may be attributed to inhibition of PDE5 by sildenafil that activates a nuclear cGMP−dependent protein kinase PKG with an overall detrimental effect on sperm chromatin structure. Furthermore, we may speculate that the effect of sildenafil on sperm DNA is due to the formation of hydrogen bonds between the C=O groups of the molecule of sildenafil and the NH2 group in the guanine moiety of the DNA. The latter hypothesis is very vividly supported by previous studies revealing this mechanism as the responsible mechanism for the interaction between sildenafil with salmon sperm DNA (Biosensors and Bioelectronics 22, 2007, 2471–2477).

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