## SCHEDULE AT A GLANCE

**American Society of Andrology 43rd Annual Conference**  
“Andrology Today, Tomorrow & Beyond - Bridging Science & Clinical Practice”  
**April 21- 24, 2018**  
The Nines | Portland, Oregon  
Program Chairs: Nina S. Davis, MD and Steven M. Schrader, PhD

All sessions will be located in **Culture/Fashion** unless otherwise noted. Speakers and times are subject to change.

### FRIDAY, APRIL 20, 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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</table>
| 4:00 p.m. - 6:00 p.m. | Registration/Information Desk Hours | The Nines Ballroom Foyer  
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### SUNDAY, APRIL 22, 2018 (Continued)

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Details</th>
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<tbody>
<tr>
<td>12:30 p.m. - 2:00 p.m.</td>
<td><strong>WOMEN IN ANDROLOGY LUNCHEON</strong> (Sponsored by the Women in Andrology Committee)</td>
<td>Location: Gallery&lt;br&gt;Advancing the Careers of Women in Urology: Is Time Really Up?&lt;br&gt;Kathleen Hwang, PhD&lt;br&gt;Brown University</td>
</tr>
<tr>
<td>2:00 p.m. - 5:15 p.m.</td>
<td><strong>SYMPOSIUM II - Reconstituting Andrologic Function after Traumatic Injury to Brain and Body</strong></td>
<td>Moderators:&lt;br&gt;Nina S. Davis, MD, FACS&lt;br&gt;<strong>Oregon Health &amp; Science University</strong>&lt;br&gt;Trainee Moderator:&lt;br&gt;Guillermo G. Lopez, MD&lt;br&gt;<strong>Wake Forest Institute Regenerative Medicine</strong></td>
</tr>
<tr>
<td>2:00 p.m. - 2:10 p.m.</td>
<td><strong>AUA LECTURE</strong> (Supported by an AUA Educational Grant)</td>
<td>Introducer: Nina S. Davis, MD, FACS&lt;br&gt;Genital Blast Injuries: Andrologic Consequences and Therapeutic Conundrums&lt;br&gt;Robert C. Dean, MD&lt;br&gt;<strong>Walter Reed National Military Medical Center</strong></td>
</tr>
<tr>
<td>3:00 p.m. - 3:40 p.m.</td>
<td><strong>Trauma Outcomes and Urogenital Health (TOUGH) Project: Initial Findings and Future Directions</strong></td>
<td>Steven J. Hudak, MD&lt;br&gt;<strong>F. Edward Hebert School of Medicine</strong></td>
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<tr>
<td>3:40 p.m. - 3:55 p.m.</td>
<td><strong>Break</strong></td>
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<tr>
<td>3:55 p.m. - 4:30 p.m.</td>
<td><strong>Neurobiology of Traumatic Brain Injury: Hypogonadism, Biomarkers and Implications for Treatment and Recovery</strong></td>
<td>Amy K. Wagner, MD&lt;br&gt;<strong>University of Pittsburgh</strong></td>
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<tr>
<td>4:30 p.m. - 5:05 p.m.</td>
<td><strong>Spermatogonial Stem Cells to Preserve and Restore Fertility</strong></td>
<td>Kyle Orwig, PhD&lt;br&gt;<strong>University of Pittsburgh</strong></td>
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<tr>
<td>5:05 p.m. - 5:15 p.m.</td>
<td><strong>Symposium Summary</strong></td>
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<tr>
<td>6:00 p.m. - 7:00 p.m.</td>
<td><strong>ASA Research Collaboration Social</strong></td>
<td>Location TBD</td>
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### MONDAY, APRIL 23, 2018

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<th>Time</th>
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<tbody>
<tr>
<td>6:30 a.m. - 6:00 p.m.</td>
<td><strong>Registration/Information Desk Hours</strong></td>
<td>Location: The Nines Ballroom Foyer</td>
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<tr>
<td>7:00 a.m. - 8:00 a.m.</td>
<td><strong>Continental Breakfast</strong></td>
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<tr>
<td>8:00 a.m. - 11:15 a.m.</td>
<td><strong>SYMPOSIUM III - Environmental Influences on Male Reproduction</strong></td>
<td>Moderators:&lt;br&gt;Janice L. Bailey, PhD&lt;br&gt;<strong>Dept. des Sciences Animales, Universite Laval, Canada</strong>&lt;br&gt;Gail S. Prins, PhD&lt;br&gt;<strong>University of Illinois at Chicago</strong>&lt;br&gt;Trainee Moderator:&lt;br&gt;Luke Simon, PhD&lt;br&gt;<strong>University of Utah</strong></td>
</tr>
<tr>
<td>8:00 a.m. - 8:10 a.m.</td>
<td><strong>Introduction to Topic</strong></td>
<td></td>
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<tr>
<td>8:10 a.m. - 9:00 a.m.</td>
<td><strong>INTERNATIONAL LECTURE</strong> (Supported by the ASA General Endowment Fund)</td>
<td>Introducer: Janice L. Bailey, PhD&lt;br&gt;Dept. des Sciences Animales, Universite Laval, Canada&lt;br&gt;Association Between Male Reproductive Disorders and Exposure to Endocrine Disrupting Chemicals&lt;br&gt;Andreas Kortenkamp, PhD&lt;br&gt;<strong>Brunel University London</strong></td>
</tr>
<tr>
<td>9:00 a.m. - 9:40 a.m.</td>
<td><strong>Endocrine Disruption Associated with Developmental Exposure to a Mixture of Hydraulic Fracturing (Fracking) Chemicals</strong></td>
<td>Susan C. Nagel, PhD&lt;br&gt;<strong>University of Missouri</strong></td>
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<tr>
<td>9:40 a.m. - 9:55 a.m.</td>
<td><strong>Break</strong></td>
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<tr>
<td>9:55 a.m. - 10:30 a.m.</td>
<td><strong>Influence of Phthalates on Sperm Epigenetics and Early-Life Development</strong></td>
<td>J. Richard Pilsner, PhD, MPH&lt;br&gt;<strong>UMass Amherst</strong></td>
</tr>
<tr>
<td>10:30 a.m. - 11:05 a.m.</td>
<td><strong>Sperm RNAs-Mediated Epigenetic Inheritance</strong></td>
<td>Wei Yan, MD, PhD&lt;br&gt;<strong>University of Nevada School of Medicine</strong></td>
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<tr>
<td>11:05 a.m. - 11:15 a.m.</td>
<td><strong>Symposium Summary</strong></td>
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<tr>
<td>11:15 a.m. - 11:30 a.m.</td>
<td><strong>Matthew P. Hardy Young Andrologist Award</strong></td>
<td>*Not CME Accredited&lt;br&gt;(Supported by the Matthew P. Hardy Endowment Fund)&lt;br&gt;2018 Recipient: Michael L. Eisenberg, MD&lt;br&gt;Presenter: Dolores J. Lamb PhD, HCLD</td>
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<tr>
<td>11:30 a.m. - 12:30 p.m.</td>
<td><strong>Poster Session II</strong></td>
<td><em>Not CME Accredited&lt;br&gt;Location: The Nines Ballroom Pre-Function</em>*</td>
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<tr>
<td>12:30 p.m. - 2:00 p.m.</td>
<td><strong>LUNCH ON OWN</strong></td>
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<tr>
<td>12:30 p.m. - 2:00 p.m.</td>
<td><strong>EDITORIAL BOARD LUNCHEON</strong></td>
<td>Location: Studio</td>
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<tr>
<td>12:30 p.m. - 2:00 p.m.</td>
<td><strong>MENTORING LUNCHEON</strong> (Sponsored by the Diversity and Trainee Affairs Committees)</td>
<td>Location: Gallery&lt;br&gt;Translational Medicine in Andrology: From Bench to Bedside&lt;br&gt;Christina Wang, MD&lt;br&gt;<strong>Harbor-UCLA Medical Center</strong></td>
</tr>
<tr>
<td>2:00 p.m. - 5:00 p.m.</td>
<td><strong>SYMPOSIUM IV - Andrology Career Development</strong></td>
<td>Moderators:&lt;br&gt;Matthew R. Marcello, PhD&lt;br&gt;<strong>Pace University</strong>&lt;br&gt;Trainee Moderator:&lt;br&gt;Bidur Paudel&lt;br&gt;<strong>University of Massachusetts, Amherst</strong></td>
</tr>
<tr>
<td>2:00 p.m. - 2:05 p.m.</td>
<td><strong>Introduction to Topic</strong></td>
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<tr>
<td>2:05 p.m. - 2:15 p.m.</td>
<td><strong>Updates from NICHD &amp; NIEHS</strong></td>
<td>Stuart B. Moss, PhD&lt;br&gt;<strong>National Institutes of Child Health &amp; Human Development</strong></td>
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</tbody>
</table>
MONDAY, APRIL 23, 2018 (Continued)

2:15 p.m. - 3:30 p.m. Breaking Abstracts Podium Session 1
3:30 p.m. - 3:45 p.m. Break
3:45 p.m. - 5:00 p.m. Breaking Abstracts Podium Session 2
5:15 p.m. - 6:30 p.m. TRAINEE-DIRECTED MINI-SYMPOSIUM
Pushing the Boundaries: Ethics in Human Gene Editing
(see page 22 for full program schedule)
6:30 p.m. - 7:00 p.m. *Presentation of Trainee Awards
*Not CME Accredited
7:00 p.m. - 8:30 p.m. Trainee Forum and Mixer
Location: Gallery Foyer

TUESDAY, APRIL 24, 2018

7:00 a.m. - 8:00 a.m. 2019 Program Committee Meeting
Location: Frank Board Room
7:00 a.m. - 6:00 p.m. Registration/Information Desk Hours
Location: The Nines Ballroom Foyer
7:00 a.m. - 8:00 a.m. Continental Breakfast
Location: The Nines Ballroom Foyer

8:00 a.m. - 11:15 a.m. SYMPOSIUM V - Semen, Sex and Viruses
Moderators:
Maria Agustina Battistone, PhD
Massachusetts General Hospital/
Harvard Medical School
Paul J. Turek, MD
The Turek Clinic
8:00 a.m. - 8:10 a.m. Introduction to Topic
8:10 a.m. - 9:00 a.m. DIVERSITY LECTURE
(Supported by the ASA Educational Endowment Fund)
The Ebola Story in West Africa 2014
Mary J. Choi, MD, MPH
LADR, US Public Health Services
9:00 a.m. - 9:40 a.m. The Zika Epidemic: Risk to Offspring and Sexual Transmission
Gabriela Paz-Bailey, MD, PhD
Centers for Disease Control and Prevention
9:40 a.m. - 9:55 a.m. Break
9:55 a.m. - 10:30 a.m. Lessons Learned from the Sexual Transmission of HIV
John T. Brooks, MD
Centers for Disease Control and Prevention
10:30 a.m. - 11:05 a.m. Where Are We With Vaccines for Ebola and Zika Viruses?
Barney S. Graham, MD, PhD
NIH/NIAID/VRC
11:05 a.m. - 11:15 a.m. Symposium Summary

11:15 a.m. - 12:15 p.m. *Poster Session III
Location: The Nines Ballroom Pre-Function
*Not CME Accredited
12:15 p.m. - 1:45 p.m. LUNCH ON OWN
1:45 p.m. - 4:45 p.m. SYMPOSIUM VI - Male Contraception
Moderator:
Mitch Eddy, PhD
Male Contraceptive Initiative
Trainee Moderator:
Maria Gracia Gervasi, PhD
University of Massachusetts
1:45 p.m. - 1:55 p.m. Introduction to Topic
1:55 p.m. - 2:50 p.m. Current Paradigms in Contraception Method Development
Daniel S. Johnston, PhD
NIH, Contraception Research Branch NICHD
2:50 p.m. - 3:20 p.m. Development of a New Non-Hormonal Male Contraceptive Drug: Update
Michael G. O’Rand, PhD
University of North Carolina
3:20 p.m. - 3:35 p.m. Break
3:35 p.m. - 4:05 p.m. Recent Advances in Reversible Male Hormonal Contraception
Stephanie T. Page, MD, PhD
University of Washington
4:05 p.m. - 4:35 p.m. *Past, Present, and Future of Vas-Occlusive Medical Devices
*Not CME Accredited
Kevin Eisenfrats, BS
Contraline, Inc.
4:35 p.m. - 4:45 p.m. Summary: Where Do We Go from Here?
4:45 p.m. - 5:45 p.m. ASA Business Meeting
7:30 p.m. - 11:00 p.m. Annual Banquet
Location: Coopers Hall Winery and Taproom
Buses depart from hotel lobby starting at 7:00 p.m.
(Not included in registration fee: ticket required)

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

On behalf of the American Society of Andrology, I welcome you to the 43rd Annual Conference! I am so excited about our program Andrology Today, Tomorrow and Beyond: Bridging Science and Clinical Practice. I want to express my deep appreciation to Program Committee co-chairs Steve Schrader and Nina S. Davis for guiding our program, and to all the Committee members for working diligently to bring us important current topics and thought leaders in Andrology.

On Saturday, April 21 we have three all-day special intensives:
• Basic Science Workshop
  Andrology in the Dish: In Vitro Tools to Study Male Reproduction
• Andrology Lab Workshop
  Sperm Morphology Classification: a Rational Algorithm Approach
• Clinical Symposium
  TRANSFormations: Andrology and Transgender Medicine

The Annual Conference program will open on Saturday evening with the Emil Steinberger Memorial Lecture, Of Mice, Pigs, Monkeys and Men: Novel Approaches to Study Germ Line Stem Cells and Spermatogenesis, presented by Ina Dobrinski, DVM, PhD from the University of Calgary. The opening reception that follows is a chance to reconnect with our colleagues.

Six half day Symposia showcase the diversity of Andrology:
• ART and Andrology
• Reconstituting Andrologic Function after Traumatic Injury to Brain and Body
• Environmental Influences on Male Reproduction
• Semen, Sex and Viruses
• Male Contraception
• Andrology Career Development with the Trainee-Directed Mini Symposium

Each symposium will highlight exciting work in basic science, translational medicine and clinical practice, showcasing the importance of collaboration between the bench and bedside, with lectures presented by international experts and thought leaders in Andrology. Moderators will introduce each session with a brief overview and sum up each session with a look to the future challenges presented by the lectures.

As always, the latest work by our attendees will be showcased in several poster and oral sessions designed to give thoughtfull discussion of new work from all over the world. Luncheons throughout the meeting provide informal presentations with our key interest groups.

A new feature of this meeting is the Research Collaboration Social, an event dedicated to connecting basic researchers and clinical practitioners in a quick "speed-date" format. Bring your ideas and collaborative needs with a 3 minute introduction to find a partner for future work together. We think this will be a fun and productive session for all.

The Annual Conference is also our opportunity to celebrate three outstanding members. Congratulations to our 2018 Distinguished Andrologist, John McCarrey, BS, MS, PhD and 2018 Matthew P. Hardy Young Andrologist, Michael Eisenberg, MD. We will present the Distinguished Service Award posthumously to Rudi Ansbacher, MD. We will present the Distinguished Service Award posthumously to Rudi Ansbacher, MD, a founding member who will be sorely missed.

During the conference, please visit our exhibitors who bring us the latest in supplies and pharmaceuticals, and who provide generous support for our meeting. The Endowment and Development Committee table has opportunities for supporting our funds in many ways. Thanks to all those who have donated gifts this year; there is still time to help make our Annual Fund goal for 2017-2018.

Our conference is headquartered in a beautiful boutique hotel, The Nines, ideally located at Pioneer Square in the central business district in one of the country’s premier walking cities. Browse nearby stores, dine in the trendy Pearl District, or use the eco-friendly MAX light rail to explore the beautiful gardens, museum grounds, and chic neighborhoods that pepper the lively city of Portland. Our Annual Banquet will close our meeting in a quintessential Portland venue, a wine and beer tasting site with live music and dancing after a great meal.

It has been my honor to serve as President this year. Thanks to my fellow officers, Executive Council members and our hard working committee chairs, we have worked on strategic issues that are vital for the future.

I am excited for you all to attend this wonderful conference in the city of Portland and I hope you leave with new ideas that will transform your research and clinical practice in Andrology, today, tomorrow and beyond.

Sincerely,

Susan A. Rothmann, PhD, HCLD
ASA President

PAST PRESIDENTS OF THE AMERICAN SOCIETY OF ANDROLOGY

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<td>2017-18</td>
<td>Matthew P. Hardy</td>
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<tr>
<td>2016-17</td>
<td>Sally Perreault Darney</td>
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<td>2014-15</td>
<td>William J. Bremner</td>
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<td>2012-13</td>
<td>Jon L. Pryor</td>
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<td>2007-08</td>
<td>Terry T. Turner</td>
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<td>2003-04</td>
<td>Richard V. Clark</td>
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<td>1999-2000</td>
<td>Barry T. Hinton</td>
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<td>1992-93</td>
<td>Ronald S. Swerdloff</td>
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<td>1991-92</td>
<td>David W. Hamilton</td>
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<td>1990-91</td>
<td>Howard Nankin</td>
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<td>1989-90</td>
<td>Rupert Amann</td>
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<td>1986-87</td>
<td>Glenn R. Cunningham</td>
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<td>1985-86</td>
<td>Marie-Claire Orgebin-Crist</td>
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<td>1984-85</td>
<td>Anna Steinberger</td>
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<td>1982-83</td>
<td>Rudi Ansbacher*</td>
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<td>1981-82</td>
<td>Richard J. Sherins</td>
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<td>1980-81</td>
<td>Philip Troen</td>
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<td>1979-80</td>
<td>Nancy J. Alexander</td>
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<td>1978-79</td>
<td>C. Alvin Paulsen*</td>
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<tr>
<td>1977-78</td>
<td>Don W. Fawcett*</td>
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<tr>
<td>1977-77</td>
<td>Emil Steinberger*</td>
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*Deceased

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Kenneth P. Roberts, PhD

Past President
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David S. Karabinus, PhD, HCLD; Montpelier, VA (Chair)

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Gail A. Cornwall, PhD; Lubbock, TX (Chair)

Basic Science Workshop
Elizabeth Snyder, PhD; New Brunswick, NJ (Chair)

Bylaws Committee
Erma Z. Drobnis, PhD, HCLD; Columbia, MO (Chair)

Clinical Symposium
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Wylie C. Hembree, MD; Woodcliff Lake, NJ (Co-Chair)

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Hooman Sadri-Ardekani, MD, PhD; Winston Salem, NC (Co-Chair)

Endowment Committee
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Wei Yan, MD, PhD; Reno, NV (Co-Chair)

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Patricia S. Cuasnicu, PhD; Buenos Aires, Argentina (Co-Chair)

Journal Oversight Committee
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Journal Editors
Douglas T. Carrell, PhD, HCLD; Salt Lake City, UT (Editors-In Chief)
Manuela Simoni, MD, PhD; Modena, Italy (Editors-In Chief)

Liaison Committee
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Local Arrangements Committee
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Peter Liu, MBBS, PhD, FRACP; Torrance, CA (Co-Chair)

Nominating Committee
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Steven M. Schrader, PhD; Cincinnati, OH (Co-Chair)

Trainee Affairs
Matthew R. Marcello, PhD; New York, NY (Chair)

Women in Andrology
Nina S. Davis, MD, FACS; Portland, OR (Chair)
Maria Christina W. Avellar, PhD; São Paulo, Brazil (Vice Chair)
Jannette Dufour, PhD; Lubbock, TX (Past Chair)

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Email: andrologysociety.org
Website: mc.manuscriptcentral.com/andrology

EXECUTIVE OFFICE
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Schaumburg, IL 60173
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Email: info@andrologysociety.org

NOTICE TO READERS
Every effort has been made to ensure the information printed here is correct; however, details are subject to change.
WELCOME TO PORTLAND
Portland’s compact, walkable downtown offers easy access to great food, green spaces, cultural offerings and tax-free shopping. Browse the city’s most diverse mix of retail brands, see a show and grab a bite at a food cart or fine restaurant.

Downtown Portland is easily accessible via car, bike or public transportation, with a mix of on-street, private and public parking garages.

Attractions/Entertainment
From the theaters and museums of the Cultural District to parks that play host to festivals and farmers’ markets, downtown puts a wide range of entertainment within easy walking distance. The long green lawns, riverside paths and refreshing fountains of Waterfront Park are a magnet for joggers, cyclists and Frisbee flingers; they also set the stage for a full slate of summer festivals.

Nicknamed Portland’s Living Room, red-brick-lined Pioneer Courthouse Square is a hub of civic fun. The most-visited spot in town hosts some 300 events each year, including a farmers’ market on summer Mondays, free concerts, movies and a grand holiday tree-lighting party.

One of Portland’s most popular attractions is Powell’s City of Books. Starting as a humble storefront in 1971, Powell’s has become a Portland landmark and one of the world’s greatest bookstores. Housing over 1 million books in 3,500 different sections, the bookstore takes up an entire city block. While you explore the largest independent used and new bookstore on Earth, be sure to stop at the in-store coffee shop for some fuel.

Another great Portland attraction is the Lan Su Chinese Garden, an authentically built Ming Dynasty style garden. Surrounding a picturesque lake are covered walkways, bridges, and a beautifully planted landscape. This peaceful urban oasis was built by artisans from Portland’s sister city of Suzhou. Tours are available and there is a teahouse that serves snacks and traditional teas.

Dining
The Washington Post claims Portland is America’s best food city, offering innovative restaurants, food carts, and more.

Want to indulge in Portland’s top cuisine? Travelportland.com lists the best new restaurants to try. Visit the highly anticipated Roe, a former pop-up offering some of the best seafood in the city, located in Southwest Portland’s downtown area. Another noteworthy new dining spot is Southfork in Northeast Portland, serving both modern and traditional Southern cuisine. It also features live jazz music on Friday and Saturday nights.

Home to more than 600 food carts, CNN declared Portland home to the world’s best street food. Grouped in “pods” all around town, it’s easy to sample multiple carts at a time. A popular pod to try is Alder pod, the city’s largest. It features carts like The Frying Scotsman, where you can try traditional British fish and chips and The Whole Bowl, which serves veggie bowls topped with garlic sauces.

To learn more about Portland activities, visit the city website at www.travelportland.com.

HOTEL INFORMATION

The Nines Portland
525 SW Morrison Street
Portland, OR 97204
Main: (877)229-9995
Website: www.thenines.com

TRAVEL AND TRANSPORTATION

Airport Information
Portland International Airport (PDX)
The airport is located 9 miles (14.5 km) northeast of downtown Portland and is conveniently connected to the city center via MAX light rail train.

The MAX light rail Red Line is the easiest way to travel to and from the airport. Here are some quick facts:
- The trip between the airport and downtown Portland takes about 40 minutes to The Nines Hotel. Exit at Pioneer Courthouse Square.
- An adult ticket costs $2.50 (Youth $1.25, Honored Citizen $1). MAX ticket machines return change in coins, so small bills are recommended.
- You can roll your luggage on board.
- The first train of the day arrives at PDX at 4:45 a.m. The last train departs PDX at 11:50 p.m.
- The MAX station and ticket machines are located on the lower level, next to the south baggage claim area (turn right at the base of the escalator).

Hotel Parking
The Nines Hotel is pleased to offer valet parking to our guests on a daily/overnight basis with unlimited access throughout the day and night. Short term rate of $15.00. Overnight rate of $47.00, $55.00 for oversized vehicles, and $35.00 for hybrid vehicles.

TRIMET PUBLIC TRANSPORTATION

TriMet Buses
TriMet buses serve much of the Portland metro area. Many bus lines connect with MAX Light Rail, WES Commuter Rail and the Portland Streetcar. Contact our concierge for a bus schedule and rates.

TriMet WES
TriMet’s WES (Westside Express Service) is a commuter rail line serving Beaverton, Tigard, Tualatin and Wilsonville. WES runs every 30 minutes during the weekday morning and afternoon rush hour.

Streetcar
The Portland Streetcar connects Northwest Portland, the Pearl District, Portland City Center, PSU, RiverPlace and the South Waterfront District. The Streetcar is owned and operated by the City of Portland.
GENERAL MEETING INFORMATION

REGISTRATION/INFORMATION DESK HOURS:
Location: The Nines Ballroom Foyer

Friday, April 20: 4:00 p.m. - 6:00 p.m.
Saturday, April 21: 7:30 a.m. - 7:30 p.m.
Sunday, April 22: 6:30 a.m. - 6:30 p.m.
Monday, April 23: 6:30 a.m. - 6:00 p.m.
Tuesday, April 24: 7:00 a.m. - 6:00 p.m.

EXHIBIT HALL HOURS:
Location: Design

Saturday, April 21: 4:00 p.m. - 9:00 p.m.
Sunday, April 22: 7:00 a.m. - 4:00 p.m.

SPECIAL EVENTS

Welcome Reception
Date: Saturday, April 21, 2018
Time: 7:30 p.m. - 9:00 p.m.
Dress: Business casual or casual attire is appropriate
Cost: One ticket included in ASA registration; $50.00 for additional tickets. Join us for a welcome reception to connect with friends and colleagues. Please sign up for this event on the registration form.

Women in Andrology Luncheon and Discussion
Date: Sunday, April 22, 2018
Time: 12:30 p.m. - 2:00 p.m.
Host: Nina S. Davis, MD, FACS
Cost: $25.00 for Trainees, $45.00 for Attendees (Member/Non-Member). Please sign up for this event on the registration form.

ASA Research Collaboration Social
Date: Sunday, April 22, 2018
Time: 6:00 p.m. - 7:00 p.m.
Location: TBD
Cost: Complimentary; all members of the society are welcome. This is an informal event with light snacks, beer, and wine provided.

Mentoring Luncheon
Sponsored by the Diversity and Trainee Affairs Committees
Translational Medicine in Andrology: From Bench to Bedside
Date: Monday, April 23, 2018
Time: 12:30 p.m. - 2:00 p.m.
Speaker: Christina Wang, MD, Harbor-UCLA Medical Center
Cost: $25.00 for Trainees, $45.00 for Attendees (Member/Non-Member). Please sign up for this event on the registration form.

Trainee Forum and Mixer
Date: Monday, April 23, 2018
Time: 7:00 p.m. - 8:30 p.m.
Cost: Complimentary; all members of the society are welcome. Please sign up for this event on the registration form.

Annual Banquet
Date: Tuesday, April 24, 2018
Time: 7:30 p.m. - 11:00 p.m.
Location: Cooper’s Hall Winery and Taproom
Cost: $80.00 for Attendees (Member/Nonmember), $40.00 for Trainees. Includes dinner and entertainment. Please sign up for this event on the registration form.

OPTIONAL WORKSHOPS/SYMPOSIA

Andrology Lab Workshop
Sperm Morphology Classification: a Rational Algorithm Approach
Date: Saturday, April 21, 2018
Time: 8:30 a.m. - 5:00 p.m.
Location: Studio
Cost: ASA Members $295.00 and Non-Member $345.00.
Please sign up for this event on the registration form. Please note the Andrology Lab Workshop includes the named “Lab Science Forum” Luncheon and Lecture.

ASA Basic Science Workshop
Andrology in a Dish: In Vitro Tools to Study Male Reproduction
Date: Saturday, April 21, 2018
Time: 8:30 a.m. - 4:30 p.m.
Location: Gallery 123
Cost: $50.00 for Attendees (Member/Nonmember).
*Lunch included in BSW registration fee.
Please sign up for this event on the registration form.

ASA Clinical Symposium
TRANSformations: Andrology and Transgender Medicine
Date: Saturday, April 21, 2018
Time: 8:00 a.m. - 5:00 p.m.
Location: Culture/Fashion
Cost: $50.00 early/$75.00 onsite for Trainees, $75.00 early/$100.00 onsite for Attendees (Member/Nonmember). This year the Clinical Symposium has been expanded from a half day program to a full day of educational lectures. Please sign up for this event on the online registration form.

Due to the subject matter, ASA is offering a special $25.00 General Public - Non-Medical/Scientific Attendee Registration - subject to ASA Business office approval.
MESSAGE FROM THE PROGRAM CO-CHAIRS

It is with great excitement and pride that we welcome you to the 43rd Annual Meeting of the American Society of Andrology and to the city of Portland, Oregon! It is our hope that you will find both the meeting and the surroundings stimulating, diverse, enriching and, most of all, informative.

This year’s program, Andrology Today, Tomorrow and Beyond—Bridging Science and Clinical Practice, represents a new format. The meeting has been expanded to a full 4 days to maximize educational content and to cover the breadth of modern Andrology. Beginning with 3 day-long symposia, the Andrology Laboratory Workshop focusing on the classification of sperm morphology, the Basic Science Workshop delving into tools useful for in vitro studies of male reproduction and the state-of-the-art Clinical Symposium covering virtually all aspects of transgender medicine, every attempt has been made to provide didactics that are relevant to the varied practices of our attendees and to set the stage for our translational theme.

Another “first” is that the Clinical Symposium will be a full day, stand-alone activity. Because the field of Transgender Medicine is a rapidly developing subspecialty encompassing endocrinologic, surgical, medical, psychological, sociological and andrologic subject matter, we have convened prominent thought leaders from across the US to present the most up-to-date developments in this exciting new medical discipline.

The importance and benefits of collaborations among basic scientists, translational scientists, and clinicians, the founding principle of our organization, is embodied in the subsequent symposia and expert lectures that fill the next 3 days of plenary sessions. The ASA meeting opens with Emil Steinberger Memorial Lecture by Ina Dobrinski providing novel approaches to study germline stem cells and spermatogenesis. The plenary sessions start with ART, first with implementation of techniques to restore the predictive value of sperm morphology in the Andrology lab followed by the emerging ART of preserving and transplanting spermatogonial stem cells. The sessions continue literally with a bang – a comprehensive review by the emerging ART of preserving and transplanting spermatogonial stem cells. The sessions continue literally with a bang – a comprehensive review of the andrologic consequences of traumatic genital and brain injuries in “wounded warriors” and the approaches being taken to mitigate and treat them. International expertise is then brought to bear on a discussion of the adverse effects of environmental toxicants – endocrine disruptors, chemicals involved in “fracking” and phthalates on male reproductive health and gamete interactions. Another threat to fertility and human development are viruses, both endemic and epidemic. Our experts will provide the latest information regarding the sexual transmission, reproductive consequences and vaccination for Ebola, Zika and HIV viruses. The search for a viable, reversible and widely applicable male contraceptive continues, and no andrology meeting would be complete without a review of the latest pharmacologic and mechanical approaches to this challenging problem.

The ASA recognizes the scientific contributions of our trainee colleagues and honors their efforts during the 2 oral sessions held during the half-day dedicated to Andrology career development. The advantage of holding a meeting in a city with a major academic institution such as Oregon Health and Science University is that proximity facilitates taking advantage of prominent local researchers and clinicians. As part of the trainee-directed activities, we have been fortunate to secure as a speaker, Dr. Kyle Orwig, who will give the keynote lecture on the ethics of gene editing using CRISPR technology during the Trainee-Directed Mini-Symposium on Monday. The Trainee Forum and Mixer that follows offers plenty of opportunity for networking and celebrating the achievements of those who will be leading the ASA forward in the future.

The breadth and quality of such a meeting would not be possible without the dedicated efforts of the Program Committee who so generously gave of their precious time and provided thoughtful guidance in honing the subject matter and securing the most accomplished speakers for the program. Their contributions are greatly appreciated. Special thanks are owed to Wylie Hembree, prominent endocrinologist and charter member of ASA and supporter, whose assistance with organizing the transgender symposium was invaluable. Gratitude is also due our President, Susan Rothmann, whose vision, energy and leadership directed and nurtured the process of creating an ambitious and comprehensive program. Finally, none of this would be possible without the support of our Executive Director, Donna Rostamian, and the staff at W.J. Weiser. Their years of service providing assistance with day-to-day affairs have been instrumental in sustaining the organization.

Although ASA 2018 offers a full agenda of symposia and lectures, we hope that you will find time to step out and partake of the myriad experiences Portland has to offer including the banquet Tuesday night at the Coopers Hall Winery and Tap Room, a quintessential Portland venue. There is no better way to end your sojourn in the City of Roses.

Steven Schrader, PhD
Nina S. Davis, MD

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CLINICAL SYMPOSIUM

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Wylie C. Hembree, MD; Woodcliff Lake, NJ (Co-Chair)

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Andrology, 2018, Supplement, 9
**EMIL STEINBERGER MEMORIAL LECTURE AWARD**

(Supported by the Emil Steinberger Endowment Fund)

**Ina Dobrinski, DVM, PhD**
*University of Calgary, Faculty of Veterinary Medicine (Canada)*

Dr. Dobrinski is the Head of the Department of Comparative Biology and Experimental Medicine. She joined the University of Calgary in 2008 after 11 years at the University of Pennsylvania, School of Veterinary Medicine, where she was a Professor of Reproduction, the Director of the Center for Animal Transgenesis and Germ Cell Research, and the Marion Dilley and Robert George Jones Chair in Reproduction.

Research in the Dobrinski laboratory is focused on mammalian germ line stem cell biology. This lab was the first to apply germ line stem cell transplantation in non-rodent models to transmit a genetic change introduced into germ line stem cells to the next generation. The work enables generation of genetically modified non-rodent animal models that is potentially more efficient than the current approaches. They also developed xenografting of testis tissue as an accessible in vivo system to study germ line stem cells and spermatogenesis. This technique makes it possible to produce sperm from immature males for the first time and allows controlled experimentation in donor species such as primates where experiments in whole animals would be logistically and ethically difficult.
DISTINGUISHED ANDROLOGIST AWARD
(Supported by the Eugenia Rosemberg Endowment Fund)

This is the highest award of the Society, presented annually to an individual who has made an outstanding contribution to the progress of Andrology.

John McCarrey, BS, MS, PhD
University of Texas at San Antonio, Department of Urology

Dr. McCarrey received his Bachelors degree in Animal Science and his Masters and PhD degrees in Genetics, all from the University of California, Davis. He did a postdoctoral fellowship with Dr. Susumu Ohno at the City of Hope in Duarte, California. He then joined the faculty in Reproductive Biology at the Johns Hopkins School of Public Health. In 1991 he moved to the Department of Genetics at the Southwest Foundation for Biomedical Research in San Antonio, Texas, and in 2001 he assumed his present position as Professor of Cell & Molecular Biology at the University of Texas at San Antonio. He holds joint appointments in the Departments of Obstetrics and Gynecology and Cellular & Structural Biology at the University of Texas Health Science Center at San Antonio, and in the Department of Comparative Medicine at the Texas Biomedical Research Institute. He is also an affiliate scientist of the Southwest National Primate Research Center in San Antonio, and Director of the San Antonio Cellular Therapeutics Institute. In 2012, Dr. McCarrey was named the Robert and Helen Kleberg Distinguished Chair in Cellular & Molecular Biology.

Dr. McCarrey’s research interests focus on the development, differentiation and function of mammalian germ cells and stem cells. He discovered the first example of a functional, germ-cell-specific retroposon in the human genome. He has published several papers on mechanisms that regulate germ-cell-specific gene expression in mammals. He has also published several papers on mechanisms of epigenetic programming that function during germ cell development and gametogenesis, and in stem cells. Additional research interests include mechanisms governing X-chromosome activity in germ cells and early embryos, mechanisms governing genetic integrity in germ cells and stem cells, the effects of cloning and assisted reproductive technologies on genetic integrity, and the development of nonhuman primate model systems for studies of stem cell research and regenerative medicine. Recently he has focused on mechanisms involved in the induction of epimutations by environmental disruptions including the use of assisted reproductive technologies, and the extent to which these are reprogrammed in the mammalian germ line. His newest interest is in the development of foundational spermatogonial stem cells in the mammalian testis.

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-Claude 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
2014 Gail S. Prins
2015 Deborah A. O’Brien
2016 Barry T. Hinton
2017 Masaru Okabe

The Distinguished Andrologist Award is sponsored by the Eugenia Rosemberg Endowment Fund.
DISTINGUISHED SERVICE AWARD
(Supported by the ASA Past Presidents Endowment Fund)

This award is bestowed annually to recognize an individual who has provided distinguished service to The American Society of Andrology.

Rudi Ansbacher, MD
University of Michigan Medical Center
October 11, 1934 - January 3, 2018

Dr. Ansbacher passed away on January 3, 2018 in Ann Arbor, MI.

Dr. Ansbacher earned his medical degree from the University of Virginia Medical School in 1959. He entered the US Army Medical Corps in 1960 and took a year of general surgery residency from 1962 to 1963 at Womack Army Hospital, Fort Bragg, North Carolina. In 1966, he completed a three year residency in obstetrics and gynecology at Letterman Army Medical Center in San Francisco. He then completed a two-year fellowship in reproductive biology at the University of Michigan Medical Center in 1971, earning a Master of Science Degree in 1970.

After 20 years of service, Dr. Ansbacher retired from the US Army as Colonel in 1980. He then became a Professor in the Department of Obstetrics and Gynecology at the University of Michigan Medical Center, in which he was granted Emeritus status in 2002.

Dr. Ansbacher established the mentorship program for Ob/Gyn residents in 1981 and instituted the mentoring of junior faculty in the department in 1996. His main research interests included those associated with female and male infertility, the immunology of reproduction, menopause, and health policy issues.

Dr. Ansbacher authored or co-authored 108 scientific papers and 18 chapters, and served as a reviewer for nine scientific journals.

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
2014  Susan Rothmann
2015  Steven M. Schrader
2016  Donna L. Vogel
2017  Janice P. Evans

Distinguished Service Award is sponsored by the ASA Past Presidents Endowment Fund.
This annual award is bestowed upon an Active Member of the American Society of Andrology who at the time of the award, is less than forty-five (45) years of age and who has made significant contributions to the field of Andrology.

Michael L. Eisenberg, MD
Stanford University School of Medicine

Michael L. Eisenberg, MD earned his bachelor degree from Rice University and his medical doctorate from Yale School of Medicine. He completed his internship and residency in urology at the University of California, San Francisco and a Male Reproductive Medicine and Microsurgery fellowship at Baylor College of Medicine in Houston, Texas. He is board certified in urology.

Dr. Eisenberg serves as an associate editor of Fertility and Sterility and Andrology, on the editorial board of the Journal of Assisted Reproduction and Genetics, and as an ad hoc referee for dozens of leading medical journals and has himself authored numerous peer-reviewed articles. His laboratory seeks to understand the association between a man’s reproductive and overall health by analyzing large clinical and administrative databases. He also has active collaborations with several groups examining pathways of spermatogenesis.
Robert C. Dean, MD
Walter Reed National Military Medical Center

Dr. Dean is a graduate (Class of 1985) of the University of Rochester, N.Y. with a Bachelor of Science degree in Cell Biology. After receiving his M.D. degree from Uniformed Services University of the Health Sciences in Bethesda, MD in 1989, he went on to complete in internship at Tripler Army Medical Center in Honolulu, HI. After several tours within the US Army Medical Command in Germany, New Mexico, Texas, and Saudi Arabia, Dr. Dean started his Urology Residency at Walter Reed Army Medical Center in Washington, DC.

Dr. Dean graduated from his Urology Residency at Walter Reed Army Medical Center in 2000. He gained further urology training in Andrology (Male Sexual Health and Infertility) at the University of California, San Francisco and completed this fellowship in 2005. Upon returning to Walter Reed, he was made the Director of Andrology. His interests include treatments for erectile dysfunction, medical management for erectile preservation, genital trauma, peyronie's disease, and male infertility. His present research projects involve chemoprevention treatments for prostate cancer, pathological analysis of prostate tissues, neurovascular preservation of erectile function and infertility preservation and sexual medicine therapies in complex trauma patients.

COL Dean’s military awards include the Bronze Star Medal, the Meritorious Service Medal with 3 Oak Leaf Clusters, the Army Commendation Medal, the Joint Service Achievement Medal, and the Army Achievement Medal with Oak Leaf Cluster. In addition, COL Dean has several military service medals awarded during Operation Iraqi Freedom and Operation Desert Falcon.
THANK YOU TO DONORS & SPONSORS

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**NOTE:** If you have included ASA in your estate planning and have not informed the Society, please consider doing so by contacting Gail Prins or Susan Rothmann. Requests for confidentiality will certainly be honored.
Creating a Legacy

The American Society of Andrology (ASA) is a professional society whose mission is to advance discovery and education in andrology, the study of male reproductive health. To maintain the health of our Society long into the future, it is essential to build a robust Endowment portfolio that supports this mission.

How are Endowment and Asset Funds Managed?

ASA is an Organizational Partner of the Cleveland Foundation, the oldest community foundation in the USA. The Foundation holds and manages our fund investments for greater return on investment and fiscally prudent growth. Each year, a percentage of funds is available for the needs of ASA. Assets are reinvested to protect against inflation and to meet the future needs of the organization.

How Can I Help the American Society of Andrology?

GIVING NOW ... Gifts of Cash or Appreciated Assets …

- These gifts are simple and immediately impactful and may be directed to any of ASA’s permanent endowment funds.
- A gift of $10,000 and above provides the donor with an opportunity to initiate a new named endowment fund (pending Council approval) & determine how its income will benefit ASA and its members. ASA will work with donors to fundraise to reach target levels for named lectureships or awards (typically $50,000+).

GIVING IN THE FUTURE … represents an opportunity to establish an enduring legacy within ASA’s permanent endowments, in many cases without impacting today’s life style.

What Long Term Gift Vehicles are Available?

ASA’s partnership with the Cleveland Foundation offers donors a variety of giving vehicles to meet their philanthropic goals. Cleveland Foundation staff will work with you, ASA and your professional advisors to find a vehicle based on your unique needs and circumstances and create sample illustrations.

Bequests: A bequest to ASA in one’s will or trust is the easiest ways to provide a future gift that benefits your special interest. Our advisors will work with your legal professional on specific bequest language.

Charitable Gift Annuities: A charitable gift annuity is an irrevocable gift that returns an income stream to the donor or another beneficiary over their lifetime. The donor qualifies for an income tax charitable deduction for part of the gift, while the payments to the beneficiary may receive favorable tax treatment. At the end of the gift term, the remainder of the annuity would support a specific ASA Endowment Fund to help substantially meet ASA’s future needs.

Life Insurance Policies: Life insurance policies can be an excellent tool to support the ASA with many different ways to structure such a gift.

Trusts: Charitable Remainder Trusts allow donors to create an income stream for themselves or other beneficiaries for a term of years, with the remainder of the trust corpus creating a gift to ASA and are flexible enough to permit the use of a variety of assets. As the opposite of a Charitable Remainder Trust, a Charitable Lead Trust provides payments to ASA and remaining assets would be transferred to other individuals or even back to the donor at a future date.

Your Legacy Gift can impact ASA for generations to come.

Visit www.andrologysociety.org to learn more about us. For more information about these or other gifting vehicles, please contact ASA’s Advancement Consultant Larry Becker at 330-696-6709 or The Cleveland Foundation Advancement Team at 877-554-5054, referencing the American Society of Andrology.
EDUCATIONAL NEEDS & OBJECTIVES

43RD ANNUAL ASA MEETING
Andrology Today, Tomorrow and Beyond – Bridging Science and Clinical Practice

COURSE DESCRIPTION
The diagnosis and management of many conditions in andrology have been greatly influenced by recent pharmacological, surgical and basic science advances. One of the greatest challenges in this discipline is to keep abreast of the many dynamic changes in this field. An internationally acclaimed faculty has been assembled to provide this update, with presentations on topics such as Standardization of Sperm Morphology, Emerging Clinical Research and Technologies for Human Spermatogonial Stem Cells, Male Contraception, Male Infertility, Viruses in Human and Transmission of viruses after Disease Recovery, Environmental Influences on Male Reproduction and Reconstituting Andrologic Function after Traumatic Injury to Brain and Body. During the plenary sessions, attendees will have the opportunity to participate in question and answer sessions.

TARGET AUDIENCE
Practicing community and academic urologists, Ph.D researchers, graduate students, andrology lab personnel, physician extenders in fertility and urology practices, DVM practitioners and candidates with a reproductive focus.

EDUCATIONAL NEEDS
There have been many recent advances in the basic science, translational science and clinical understanding of male reproductive health. Urologists, basic, and translational scientist should be up-to-date on the latest advances, research efforts, and treatment recommendations regarding conditions such as traumatic injury, contraception, viral infections transmitted in semen, effects of environmental exposures on male reproductive health, the latest advance in reproductive technologies, and the clinical treatment of transgendered individuals. Reproductive urologist patients present with the relevant concerns and conditions. These practitioners need to be updated on the advances in diagnostic modalities and treatment options for these conditions. Many collaborating Ph.D researchers working in reproductive biology and cell biology do not have an awareness of the clinical management of these conditions, their relatively high prevalence, and the need for an increased understanding of underlying biology of these conditions. Researchers will benefit from awareness of the epidemiology of these conditions, both in terms of their commonality and their predisposition to other urologic and general disease. Furthermore, an understanding of the epidemiologic impact of the treatment for these conditions (in terms of risk to offspring) is vital.

An awareness of future directions for research is useful to the audience as well and updated reviews will help identify future targets for research and novel treatments in fertility medicine.

A review of these topics will prove hugely useful to urologists and other MDs, PhD researchers, DVMs and trainees as well as physician extenders in andrology and laboratory professionals working in fertility medicine.

EDUCATIONAL OBJECTIVES:
At the conclusion of the ASA 43rd Annual Conference, attendees will be able to:
1. Explain the significance of sperm morphology to fertility outcomes.
2. Describe the history of sperm morphology classification.
3. Identify the problem of overly stringent classification of morphologically normal sperm.
4. Learn a method of classification that is rational, repeatable and defined.
5. Prescribe to become less strict in classification and reproduce WHO 5 reference values.
6. Restore predictive value in sperm morphology.
7. Present the types and sources of blast injuries relevant to male reproductive health.
8. Discuss the challenges involved in treating these injuries.
9. Review current and future areas of research in the prevention and treatment of traumatic blast injuries.
10. Describe the purpose and goals of the TOUGH Project.
11. Analyze the data that has been collected from the TOUGH project thus far and its significance with respect to andrologic effects and outcomes; outline the protocols that have been developed in response to the findings of the study.
12. Discuss the central effects of TBI as they relate to hormonal perturbations and male reproductive potential and describe interventions being implemented on the basis of this knowledge.
13. Outline future areas of research to address the acute and chronic effects of TBI on male genital health and reproductive capacity.
14. Outline the challenges in maintaining or restoring fertility in the face of severe testicular injury and tissue loss.
15. Describe currently available means of preserving testis tissue.
16. Describe current and future efforts to create germ cells from stem cell precursors.
17. Discuss male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union.
18. Demonstrate the feasibility of mixture risk assessment using data from 67 pesticides in a joint FAO/WHO case study.
19. Discuss the effects of common pesticides on prostaglandin D2 inhibition and COX-2 activity in mouse Sertoli cells.
20. Analyze the data that asks whether we can explain declining male reproductive health with known androgens.
21. Analyze the association between oil and natural gas extraction processes and human reproduction.
22. Demonstrate estrogen and androgen receptor activities of hydraulic fracturing chemicals and surface and ground water in drilling-dense regions.
23. Explain how endocrine-disrupting activity of hydraulic fracturing chemicals adversely affects health outcomes after prenatal exposure in male mice.
24. Examine evidence for environmental susceptibility of the sperm epigenome during windows of male germ cell development.
26. Describe the types and roles of various non-coding RNAs groups.
27. Describe how sperm-borne miRNAs and endo-siRNAs are important for fertilization and pre-implantation embryonic development.
28. Examine evidence for the influence of pesticide exposure (vinclozolin) on sperm small noncoding RNAs and their role in environmental transgenerational inheritance.
29. Review the clinical and epidemiological features of the current Zika outbreak and associated birth defect issues.
30. Discuss the current understanding of the sexual transmission of Zika and the CDC recommendations for travelers.
31. Delineate the basic science (mouse model) knowledge of Zika and male infertility (testis atrophy and persistence of virus in semen).
32. Review the current understanding of the mechanism and efficiency of HIV sexual transmission.
33. Discuss how sexual transmission of Zika and Ebola viruses are similar and different from that of HIV.
34. Explain the scientific strategies for developing Ebola virus vaccines.
35. Explain the scientific strategies for developing Zika virus vaccines.
36. Identify current research and clinical trials for Ebola and Zika virus vaccines.

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EDUCATIONAL NEEDS & OBJECTIVES

2018 ASA CLINICAL SYMPOSIUM
TRANSformations: Andrology and Transgender Medicine

EDUCATIONAL NEEDS:
With an increased appreciation of the prevalence of gender dysphoria or gender-incongruent individuals in the pediatric and adult populations in the United States and beyond, a number of multidisciplinary programs have been established to provide the necessary gender-affirming medical, psychiatric and surgical care that those affected require to successfully transition to their desired gender. The rapid development of the field of Transgender Medicine has not permitted practitioners not directly involved in transgender care to acquire an understanding of the endocrinologic and surgical interventions critical to successful transition. This in-depth seminar covering the latest iteration of the Endocrine Society’s Clinical Practice Guideline as well as state-of-the-art gender-affirming surgical procedures will impart a solid understanding of all aspects of transgender care, as it is anticipated that many urologists, general practitioners, internists, endocrinologists and others not previously exposed to transgender medicine will have clinical interactions with transgender individuals in the future.

As part of the preparation to transition, there is often a desire to maintain fertility potential which requires obtaining and freezing gametes for later use in fertility procedures. How this is accomplished is of interest to those in the andrologic community and will be addressed during the course of the symposium.

EDUCATIONAL OBJECTIVES
At the conclusion of the ASA Clinical Symposium, attendees should be able to:
1. Review the terminology of transgender medicine.
2. Define gender dysphoria and how it is diagnosed.
3. Outline endocrine considerations in abetting transition from adolescence to advanced adulthood.
4. Describe the preparations needed before gender-affirming procedures can be performed in Male to Female (MTF) and Female to Male (FTM) transition.
5. Describe the various gender-affirming procedures, complications and outcomes.
6. Explain the lack of correlation between gender identity and sexual preference.
7. Explore key aspects of transgender sexuality.
8. Identify the long-term medical needs of transgender individuals.

ACCREDITATION

Category 1
Creighton University Health Sciences Continuing Education designates this live activity for a maximum of 25.50 AMA PRA Category 1 Credits™. Physicians should claim only credit commensurate with the extent of their participation in this activity.

AAPA accepts AMA category 1 credit for the PRA from organizations accredited by ACCME.

ACCREDITATION STATEMENT
In support of improving patient care, this activity has been planned and implemented by Creighton University Health Sciences Continuing Education (HSCE) and the American Society of Andrology (ASA). Creighton University Health Sciences Continuing Education (HSCE) is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC), to provide continuing education for the healthcare team.

Nurses and other healthcare professionals will receive a Certificate of Attendance. For information on the applicability and acceptance of Certificates of Attendance for educational activities certified for AMA PRA Category 1 Credit™ from organizations accredited by the ACCME, please consult your professional licensing board.

DISCLOSURE REPORT
The disclosure report for this meeting will be provided to all attendees electronically prior to the start of the meeting.

If you require a printed disclosure report, please visit the registration desk.

General Disclaimer
The statements and opinions contained in this program are solely those of the individual authors and contributors and not of the American Society of Andrology. The appearance of the advertisements is not a warranty, endorsement, or approval of the products or services advertised or of their effectiveness, quality, or safety. The content of this publication may contain discussion of off-label uses of some of the agents mentioned. Please consult the prescribing information for full disclosure of approved uses. The American Society of Andrology disclaims responsibility for any injury to persons or property resulting from any ideas or products referred to in the abstracts or advertisements.

Special Assistance
We encourage participation by all individuals. If you have a disability, advance notification of any special needs will help us better serve you. Call (847) 619-4909 if you require special assistance to fully participate in the meeting.
ASA 44th Annual Conference
April 2 - 9, 2019
The Ritz-Carlton Chicago  |  Chicago, IL

REGISTRATION INFORMATION COMING SOON!

Check the ASA website for updates: www.andrologysociety.org

HOTEL INFO:
The Ritz-Carlton Chicago  |  160 E. Pearson Drive
Chicago, IL 60611  |  Main: (312) 573-3414
www.ritzcarlton.com/en/hotels/chicago

Room Rate: $215.00
Hotel Deadline: March 13, 2019
Reservations: (800) 526-2008
SCHEDULE OF EVENTS

American Society of Andrology 43rd Annual Conference
“Andrology Today, Tomorrow & Beyond - Bridging Science & Clinical Practice”
April 21 - 24, 2018
The Nines | Portland, Oregon

Program Chairs: Nina S. Davis, MD and Steven M. Schrader, PhD

All sessions will be located in Culture/Fashion unless otherwise noted. Speakers and times are subject to change.

FRIDAY, APRIL 20, 2018

4:00 p.m. - 6:00 p.m.  Registration/Information Desk Hours
Location: The Nines Ballroom Foyer

SATURDAY, APRIL 21, 2018

7:30 a.m. - 7:30 p.m.  Registration/Information Desk Hours
Location: The Nines Ballroom Foyer

4:00 p.m. - 9:00 p.m.  Exhibit Hall Open
Location: Design

8:00 a.m. - 5:00 p.m.  ASA Clinical Symposium
Location: Culture/Fashion
(See page 24 for full program schedule)

8:30 a.m. - 4:30 p.m.  *ASA Basic Science Workshop
Location: Gallery 123
(See page 25 for full program schedule)
*Not CME Accredited

8:30 a.m. - 5:00 p.m.  *ASA Andrology Lab Workshop
Location: Studio
(See page 26 for full program schedule)
*Approved for ABB Accreditation

6:00 p.m. - 6:10 p.m.  President's Welcome
Susan A. Rothmann, PhD, HCLD

6:10 p.m. - 6:30 p.m.  *ASA Distinguished Andrologist Award
*Not CME Accredited
(Supported by the Eugenia Rosenberg Endowment Fund)
2018 Recipient: John McCrery, BS, MS, PhD
Presenter: Wei Yan, MD, PhD

6:30 p.m. - 7:30 p.m.  EMIL STEINBERGER MEMORIAL LECTURE
(Supported by the Emil Steinberger Endowment Fund)
Introducer: Susan A. Rothmann, PhD, HCLD
Fertility Solutions, Inc.
Of Mice (Pigs, Monkeys) and Men:
Animal Models to Study Germline Stem Cells and Spermatogenesis
Ina Dobrinski, DVM, PhD
University of Calgary, Faculty of Veterinary Medicine (Canada)

7:30 p.m. - 9:00 p.m.  Welcome Reception in Exhibit Hall
Location: Design

SUNDAY, APRIL 22, 2018

6:30 a.m. - 8:00 a.m.  Past Presidents’ Breakfast
Location: Lobby Wine Room

6:30 a.m. - 6:30 p.m.  Registration/Information Desk Hours
Location: The Nines Ballroom Foyer

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

7:00 a.m. - 8:00 a.m.  Continental Breakfast in Exhibit Hall

8:00 a.m. - 11:15 a.m.  SYMPOSIUM I - ART and Andrology
Moderators:
Christopher Barratt, PhD
University of Dundee, United Kingdom
Christopher J. De Jonge, PhD, HCLD
University of MN Medical Center
Trainee Moderator:
Ryan Flamnigan, MD
Weill Cornell Medicine

8:00 a.m. - 9:00 a.m.  WOMEN IN ANDROLOGY LECTURE
(Supported by the Women in Andrology Endowment Fund)
Introducer: Gail S. Prins, PhD
University of Illinois at Chicago
Restoring Predictive Value of Sperm Morphology
Susan A. Rothmann, PhD, HCLD
Fertility Solutions, Inc.

9:00 a.m. - 9:40 a.m.  Characterizing Human SSCs for Transplantation
Brian P. Hermann, PhD
University of Texas at San Antonio

9:40 a.m. - 9:55 a.m.  Break

9:55 a.m. - 10:30 a.m.  Human Spermatogonial Stem Cells (hSSCs) and Fertility Preservation – Lab Requirements and the Interface Between Clinic and Lab
Hooman Sadri-Ardekani, MD, PhD
Wake Forest School of Medicine

10:30 a.m. - 11:05 a.m.  Restoration of Fertility After hSSC Transplantation - Clinical Considerations
Ellen Goossens
Vrije Universiteit Brussel, Belgium

11:05 a.m. - 11:15 a.m.  Symposium Summary

11:15 a.m. - 11:30 a.m.  *ASA Distinguished Service Award
*Not CME Accredited
(Supported by the ASA Past Presidents Endowment Fund)
2018 Recipient: Rudi Ansbacher
Presenter: Gail S. Prins, PhD

11:30 a.m. - 12:30 p.m.  *Poster Session I
Location: The Nines Ballroom PreFunction
*Not CME Accredited

12:30 p.m. - 2:00 p.m.  LUNCH ON OWN
SCHEDULE OF EVENTS

SUNDAY, APRIL 22, 2018 (Continued)

12:30 p.m. - 2:00 p.m. | WOMEN IN ANDROLOGY LUNCHEON
(Sponsored by the Women in Andrology Committee)
Location: Gallery
Advancing the Careers of Women in Urology: Is Time Really Up?
Kathleen Hwang, PhD
Brown University

2:00 p.m. - 5:15 p.m. | SYMPOSIUM II - Reconstituting Andrologic Function after Traumatic Injury to Brain and Body
Moderator:
Nina S. Davis, MD, FACS
Oregon Health & Science University
Trainee Moderator:
Guillermo G. Lopez, MD
Wake Forest Institute Regenerative Medicine

2:00 p.m. - 2:10 p.m. | Introduction to Topic
2:10 p.m. - 3:00 p.m. | AUA LECTURE
(Supported by an AUA Educational Grant)
Introducer: Nina S. Davis, MD, FACS
Genital Blast Injuries: Andrologic Consequences and Therapeutic Conundrums
Robert C. Dean, MD
Walter Reed National Military Medical Center

3:00 p.m. - 3:40 p.m. | Trauma Outcomes and Urogenital Health (TOUGH) Project: Initial Findings and Future Directions
Steven J. Hudak, MD
F. Edward Hebert School of Medicine

3:40 p.m. - 3:55 p.m. | Break
3:55 p.m. - 4:30 p.m. | Neurobiology of Traumatic Brain Injury: Hypogonadism, Biomarkers and Implications for Treatment and Recovery
Amy K. Wagner, MD
University of Pittsburgh

4:30 p.m. - 5:05 p.m. | Spermatogonial Stem Cells to Preserve and Restore Fertility
Kyle Orwig, PhD
University of Pittsburgh

5:05 p.m. - 5:15 p.m. | Symposium Summary
6:00 p.m. - 7:00 p.m. | ASA Research Collaboration Social
Location: TBA

MONDAY, APRIL 23, 2018

6:30 a.m. - 6:00 p.m. | Registration/Information Desk Hours
Location: The Nines Ballroom Foyer

7:00 a.m. - 8:00 a.m. | Continental Breakfast

8:00 a.m. - 11:15 a.m. | SYMPOSIUM III - Environmental Influences on Male Reproduction
Moderators:
Janice L. Bailey, PhD
Dept. des Sciences Animales,
Universite Laval, Canada
Gail S. Prins, PhD
University of Illinois at Chicago
Trainee Moderator:
Luke Simon, PhD
University of Utah

8:00 a.m. - 8:10 a.m. | Introduction to Topic
8:10 a.m. - 9:00 a.m. | INTERNATIONAL LECTURE
(Supported by the ASA General Endowment Fund)
Introducer: Janice L. Bailey, PhD
Dept. des Sciences Animales,
Universite Laval, Canada
Association Between Male Reproductive Disorders and Exposure to Endocrine Disrupting Chemicals
Andreas Kortenkamp, PhD
Brunel University London

9:00 a.m. - 9:40 a.m. | Endocrine Disruption Associated with Developmental Exposure to a Mixture of Hydraulic Fracturing (Fracking) Chemicals
Susan C. Nagel, PhD
University of Missouri

9:40 a.m. - 9:55 a.m. | Break
9:55 a.m. - 10:30 a.m. | Influence of Phthalates on Sperm Epigenetics and Early-Life Development
J. Richard Pilsner, PhD, MPH
UMass Amherst

10:30 a.m. - 11:05 a.m. | Sperm RNAs-Mediated Epigenetic Inheritance
Wei Yan, MD, PhD
University of Nevada School of Medicine

11:05 a.m. - 11:15 a.m. | Symposium Summary
11:15 a.m. - 12:15 p.m. | *Poster Session II
Location: The Nines Ballroom Pre-Function
*Not CME Accredited
2018 Recipient: Michael L. Eisenberg, MD
Presenter: Dolores J. Lamb PhD, HCLD

12:30 p.m. - 2:00 p.m. | LUNCH ON OWN

12:30 p.m. - 2:00 p.m. | EDITORIAL BOARD LUNCHEON
Location: Studio

12:30 p.m. - 2:00 p.m. | MENTORING LUNCHEON
(Sponsored by the Diversity and Trainee Affairs Committees)
Location: Gallery
Translational Medicine in Andrology: From Bench to Bedside
Christina Wang, MD
Harbor-UCLA Medical Center

2:00 p.m. - 5:00 p.m. | SYMPOSIUM IV - Andrology Career Development
Moderators:
Matthew R. Marcello, PhD
Pace University
Trainee Moderator:
Bidur Paudel
University of Massachusetts, Amherst

2:00 p.m. - 2:05 p.m. | Introduction to Topic
2:05 p.m. - 2:15 p.m. | Updates from NICHD & NIEHS
Stuart B. Moss, PhD
National Institutes of Child Health & Human Development

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Andrology, 2018, Supplement, 21
MONDAY, APRIL 23, 2018 (Continued)

2:15 p.m. - 3:30 p.m.  Breaking Abstracts Podium Session 1
Moderator:
Matthew R. Marcello, PhD
Pace University
Trainee Moderator:
Bidur Paudel
University of Massachusetts

2:15 p.m.
CRISPR-CAS9 MEDIATED KNOCKDOWN OF ODF2
IN PORCINE TESTICULAR SOMATIC CELLS
Presented by: Taylor Goldsmith, BsC
University of Calgary

2:30 p.m.
INVESTIGATING ZIKA VIRUS PATHOGENESIS
ON MALE REPRODUCTION USING A HUMAN
3-DIMENSIONAL TESTICULAR ORGANOID MODEL
Presented by: Nima Pourhabibi Zarandi, MD
Wake Forest Institute for Regenerative Medicine

2:45 p.m.
IN VITRO CULTURE OF HUMAN KLINEFELTER
SPERMATOGONIAL STEM CELLS
Presented by: Guillermo Galdon, MD
Wake Forest Institute for Regenerative Medicine

3:00 p.m.
PREGNANCY ESTABLISHED AFTER AUTOLOGOUS
GRAFTING OF CRYOPRESERVED TESTICULAR TISSUE
FROM PREPUBERTAL RHESUS MACAQUES
Presented by: Adetunji Fayomi, DVM, MVSc
University of Pittsburgh School of Medicine

3:15 p.m.
PUBERTAL DEVELOPMENT AFTER TESTICULAR
TISSUE BIOPSY FOR FERTILITY PRESERVATION
Presented by: Aude Braye, PhD-student
Vrije Universiteit Brussel

3:30 p.m. - 3:45 p.m.  Break

3:45 p.m. - 5:00 p.m.  Breaking Abstracts Podium Session 2
Moderator:
Elizabeth Snyder, PhD
Rutgers University
Trainee Moderator:
Taylor Kohn, MPhil
John Hopkins University

3:45 p.m.
A MOUSE GOLGIN PROTEIN, GAMP-210 IS REQUIRED
FOR ACROSOME FORMATION BUT NOT FLAGELLA
FORMATION
Presented by: Suheng Ma
Virginia Commonwealth University

3:30 p.m. 4:00 p.m.
NEW INSIGHTS INTO THE VITAMIN D
CONCENTRATION AND ITS ASSOCIATION WITH
SEmen Quality in Male Subjects
Presented by: Inari Ciccone, MsC Student
Androscience \ University of Sao Paulo

4:45 p.m.
MALIGNANCY NEGATIVELY ASSOCIATED WITH
BASELINE SEMEN PARAMETERS AMONG SPERM
BANKERS
Presented by: Rena Xu, MD, MBA
Massachusetts General Hospital

4:15 p.m.
A MOUSE GOLGIN PROTEIN, GAMP-210 IS REQUIRED
FOR ACROSOME FORMATION BUT NOT FLAGELLA
FORMATION
Presented by: Suheng Ma
Virginia Commonwealth University

5:15 p.m. - 6:30 p.m.
*4TH ANNUAL TRAINEE-DIRECTED MINI-SYMPOSIUM
"Pushing the Boundaries: Ethics in Human Gene Editing”
*Not CME Accredited
Program Co-Chairs: Parag Parekh, PhD and Nima Zarandi, MD

5:15 p.m. - 5:20 p.m.  Opening Remarks
Parag Parekh, PhD (Program Co-Chair)
Nima Zarandi, MD (Program Co-Chair)

5:20 p.m. - 5:50 p.m.  Introductory Presentations
Invited Speakers:
Kyle Orwig, PhD
Center for Embryonic Cell and Gene Therapy,
OHSU
Brian Cwik, PhD
Department of Philosophy, Portland State
University
Darius Paduch, MD, PhD
Department of Urology, Weill Cornell Medicine

5:50 p.m. - 6:20 p.m.  Q&A Session

6:20 p.m. - 6:30 p.m.  Closing Remarks
Matthew R. Marcello, PhD
Pace University

6:30 p.m. - 7:00 p.m.  *Presentation of Trainee Awards
All trainee Travel Awards and Onsite Poster
Awards will be distributed
*Not CME Accredited

7:00 p.m. - 8:30 p.m.  Trainee Forum and Mixer
Location: Gallery Foyer
## SCHEDULE OF EVENTS

### TUESDAY, APRIL 24, 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tr>
<td>7:00 a.m. - 8:00 a.m.</td>
<td>2019 Program Committee Meeting</td>
<td>Frank Board Room</td>
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<td>7:00 a.m. - 6:00 p.m.</td>
<td>Registration/Information Desk Hours</td>
<td>The Nines Ballroom Foyer</td>
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<td>7:00 a.m. - 8:00 a.m.</td>
<td>Continental Breakfast</td>
<td>The Nines Ballroom Foyer</td>
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<td>8:00 a.m. - 11:15 a.m.</td>
<td>SYMPOSIUM V - Semen, Sex and Viruses</td>
<td>The Turek Clinic</td>
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<td>Moderators: Paul J. Turek, MD</td>
<td>Henning W. Witty, MD</td>
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<td>Trainee Moderators: Maria Agustina Battistone, PhD</td>
<td>Maria Gracia Gervasi, PhD</td>
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<td>8:00 a.m. - 8:10 a.m.</td>
<td>Introduction to Topic</td>
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<td>8:10 a.m. - 9:00 a.m.</td>
<td>DIVERSITY LECTURE (Supported by the ASA Educational Endowment Fund)</td>
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<td>The Ebola Story in West Africa 2014</td>
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<td>Mary J. Choi, MD</td>
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<td>LCDR, US Public Health Services</td>
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<td>9:00 a.m. - 9:40 a.m.</td>
<td>The Zika Epidemic: Risk to Offspring and Sexual Transmission</td>
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<td>Gabriela Paz-Bailey, MD</td>
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<td>Centers for Disease Control and Prevention</td>
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<td>9:40 a.m. - 9:55 a.m.</td>
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<td>9:55 a.m. - 10:30 a.m.</td>
<td>Lessons Learned from the Sexual Transmission of HIV</td>
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<td>John T. Brooks, MD</td>
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<td>Centers for Disease Control and Prevention</td>
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<td>10:30 a.m. - 11:05 a.m.</td>
<td>Where Are We With Vaccines for Ebola and Zika Viruses?</td>
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<td>Barney S. Graham, MD</td>
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<td>NIH/NIAID/VRC</td>
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<td>11:05 a.m. - 11:15 a.m.</td>
<td>Symposium Summary</td>
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<td>1:15 a.m. - 12:15 p.m.</td>
<td>*Poster Session III</td>
<td>The Nines Ballroom Pre-Function</td>
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<td>12:15 p.m. - 1:45 p.m.</td>
<td>LUNCH ON OWN</td>
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<td>1:45 p.m. - 4:45 p.m.</td>
<td>SYMPOSIUM VI - Male Contraception</td>
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<td>Moderator: Mitch Eddy, PhD</td>
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<td>Male Contraceptive Initiative</td>
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<td>Trainee Moderator: Maria Gracia Gervasi, PhD</td>
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<td>1:45 p.m. - 1:55 p.m.</td>
<td>Introduction to Topic</td>
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<td>1:55 p.m. - 2:50 p.m.</td>
<td>Current Paradigms in Contraception</td>
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<td>Method Development</td>
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<td>Daniel S. Johnston, PhD</td>
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<td>NIH, Contraception Research Branch NICHD</td>
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<td>2:50 p.m. - 3:20 p.m.</td>
<td>Development of a New Non-Hormonal Male Contraceptive Drug: Update</td>
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<td>Michael G. O’Rand, PhD</td>
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<td>University of North Carolina</td>
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<td>3:20 p.m. - 3:35 p.m.</td>
<td>Break</td>
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<td>3:35 p.m. - 4:05 p.m.</td>
<td>Recent Advances in Reversible Male Contraception</td>
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<td>Stephanie T. Page, MD</td>
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<td>University of Washington</td>
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<td>4:05 p.m. - 4:35 p.m.</td>
<td>*Past, Present, and Future of Vas-Occlusive Medical Devices</td>
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<td>Kevin Eisenfrats, BS</td>
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<td>Contraline, Inc.</td>
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<td>4:35 p.m. - 4:45 p.m.</td>
<td>Summary: Where Do We Go from Here?</td>
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<td>4:45 p.m. - 5:45 p.m.</td>
<td>ASA Business Meeting</td>
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<td>7:30 p.m. - 11:00 p.m.</td>
<td>Annual Banquet</td>
<td>Coopers Hall Winery and Taproom</td>
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<td>*Not included in registration fee: ticket required</td>
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### DISCLAIMER STATEMENT

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

ASA Clinical Symposium
“TRANSformations: Andrology and Transgender Medicine”
SATURDAY, APRIL 21, 2018
8:00 a.m. - 5:00 p.m.
Location: Culture/Fashion
Program Co-Chairs: Nina S. Davis, MD and Wylie C. Hembree, MD

8:00 a.m. - 8:05 a.m. Welcome and Introduction
Nina S. Davis, MD
Oregon Health & Science University

8:05 a.m. - 8:25 a.m. Historical Perspective
Wylie C. Hembree, MD

8:25 a.m. - 8:45 a.m. Talking the Talk: The Language of Transgender Medicine
Amy Penkins, MSW, LCSW
Oregon Health and Science University

8:45 a.m. - 9:25 a.m. Transgender Medicine: Etiology, Epidemiology and Endocrinology
Vin Tangpricha, MD, PhD
Emory Healthcare

9:25 a.m. - 10:10 a.m. Considerations in the Treatment of the Pediatric and Adolescent Gender-Nonconforming Patient
Johanna Olson-Kennedy, MD
Children’s Hospital, Los Angeles

10:10 a.m. - 10:25 a.m. Break

10:25 a.m. - 10:55 a.m. Treating Gender Dysphoria with a Clinical Practice Guideline
Wylie C. Hembree, MD

10:55 a.m. - 11:35 a.m. Gender Identity and Sexuality
Katie Spenser, PhD
University of Minnesota

11:35 a.m. - 12:00 p.m. Q&A Session

12:00 p.m. - 1:30 p.m. Lunch & Presentation

12:15 p.m. - 12:55 p.m. Gender Dysphoria Case Studies Recognition, Evaluation and Intervention
Vin Tangpricha, MD, PhD
Emory Healthcare

1:30 p.m. - 1:50 p.m. Trans Male Bottom Surgery – Gynecology
Lishiana Shaffer, MD
Oregon Health and Science University

1:50 p.m. - 2:30 p.m. Trans Male Bottom Surgery – Phalloplasty & Metoidioplasty
Jens Berli, MD
Oregon Health & Science University

2:30 p.m. - 3:00 p.m. Trans Female Bottom Surgery
Daniel D. Dugi, III, MD, FACS
Oregon Health & Science University

3:00 p.m. - 3:15 p.m. Break

3:15 p.m. - 3:45 p.m. Sexuality and Function FTM
Joseph P. Alukal, MD
NYU School of Medicine

3:45 p.m. - 4:20 p.m. Challenges of Long-term Monitoring
Vin Tangpricha, MD, PhD
Emory Healthcare

4:20 p.m. - 4:50 p.m. Q&A Session

4:50 p.m. - 5:00 p.m. WRAP UP - Where Next?
Wylie C. Hembree, MD
SCHEDULE OF EVENTS

*ASA Basic Science Workshop
“Andrology in a Dish: In Vitro Tools to Study Male Reproduction”
SATURDAY, APRIL 21, 2018
8:30 a.m. - 4:30 p.m.
Location: Gallery 123
Program Chair: Elizabeth Snyder, PhD
*Not CME Accredited

8:30 a.m. - 8:45 a.m. Welcome and Introduction
BSW Chair: Elizabeth Snyder, PhD
Rutgers University

8:40 a.m. - 12:00 pm. SESSION I: The Power of One – Primary Cell Cultures and Cell Lines in Male Reproduction Studies

GERM CELL MODELS
Chair: Nima P. Zarandi, MD

8:45 a.m. - 9:25 a.m. Culturing Spermatogonial Stem Cells to Advance Male Reproduction
Faculty: Christopher Payne, PhD
Northwestern University

9:25 a.m. - 9:40 a.m. ORAL ABSTRACT 1
Human Spermatogonial Stem Cell Culture Using Tissue-derived Extracellular Matrices
Presented By: Sherin David
Magee-Women’s Research Institute

9:40 a.m. - 9:55 a.m. ORAL ABSTRACT 2
Development of Spermatogenesis in Three-dimension Culture From Spermatogonial Cells of Busulfan-treated Immature Mice
Presented By: Mahmoud Huleihel, PhD
Ben-Gurion University

9:55 a.m. - 10:10 a.m. ORAL ABSTRACT 3
Using the Team-2 Human Seminoma Cell Line to Study Cell Signaling Pathways Essential for Male Fertility
Presented By: Katherine Loveland, PhD
Monash University and Hudson Institute of Medical Research Australia

10:10 a.m. - 10:40 a.m. Coffee Break

SOMATIC CELL MODELS

10:40 a.m. - 11:20 a.m. Sertoli Cell Cultures
Faculty: Thomas Garcia, PhD
Baylor College of Medicine

11:20 a.m. - 11:35 a.m. ORAL ABSTRACT 4
The Role of ADCY2 Copy Number Variants in Congenital Genitourinary Anomalies
Presented By: Marisol A. O’Neill, MS
Baylor College University

11:35 a.m. - 11:50 a.m. ORAL ABSTRACT 5
In Vivo and In Vitro Effects of the Endocrine Disruptors Genistein and Mono-(2-ethylhexyl) Phthalate (Mehp) on Rodent Macrophage Inflammatory Responses
Presented By: Vanessa Brouard, PhD
University of Southern California

12:00 p.m. - 1:00 p.m. LAB SCIENCE FORUM LUNCHEON
“Semen Analysis and Sperm Epigenetic Signatures: Diagnostic Potential”
Timothy Jenkins, PhD
University of Utah

1:00 p.m. - 1:15 p.m. SESSION II: From One to Many - Tissue Explants and Organoids as Models for Male Reproduction

TESTIS EXPLANTS AND ORGANOIDS

1:00 p.m. - 1:40 p.m. Testicular Organoid: A New In Vitro Model of Male Reproduction
Faculty: Hooman Sadri-Ardekani, MD, PhD
Wake Forest University

1:40 p.m. - 1:55 p.m. ORAL ABSTRACT 6
Klinefelter Testicular Cells Culture From 2D to 3D System
Presented By: Guillermo Galdon, MD
Wake Forest School of Medicine

1:55 p.m. - 2:10 p.m. ORAL ABSTRACT 7
In Vitro Tubule and Organoid Formation Using Porcine Testicular Cells
Presented By: Taylor M. Goldsmith, BSc
University of Calgary, Canada

2:10 p.m. - 2:25 p.m. ORAL ABSTRACT 8
Sperm Production in De Novo Formed Seminiferous Tubules From Allogeneic Transplanted Testicular Cells in Rhesus Monkey Testis
Presented By: Gunapala Shetty, PhD
University of Texas M.D. Anderson Cancer Center

2:25 p.m. - 2:40 p.m. Coffee Break

REPRODUCTIVE TRACT CULTURES

2:40 p.m. - 3:20 p.m. Combining In Vitro and In Vivo Approaches to Study the Epididymis
Faculty: Clemence Belleannée, PhD
CRCUH-Q-Laval University, Canada

3:20 p.m. - 4:00 p.m. Wolffian Duct Organotypic Culture as a Tool to Study Mechanisms Underlying Epididymal Development and Morphogenesis
Faculty: Maria Christina W. Avellar, PhD
Universidade Federal de Sao Paulo - Escola Paulista de Medicina Brazil
Faculty: Barry T. Hinton, PhD
University of Virginia

4:00 p.m. - 4:15 p.m. ORAL ABSTRACT 9
Corporal Cavernosal Smooth Muscle Cell Culture as a Tool for Studying the Mechanism of Action of Pro-Erectogenic Products
Presented By: Monica Ferrini, PhD
Charles R. Drew University

4:15 p.m. - 4:30 p.m. Closing Remarks
Elizabeth Snyder, PhD
Rutgers University
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<tr>
<th>Time</th>
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<th>Speaker(s)</th>
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<tr>
<td>8:30 a.m. - 8:35 a.m.</td>
<td>Introduction</td>
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<td>Session Chair: Anna-Marie Bort, MLT(ASCP)CME</td>
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<td>8:35 a.m. - 8:45 a.m.</td>
<td>Technology Setup</td>
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<td>8:45 a.m. - 9:15 a.m.</td>
<td>Baseline Assessment of Current Methods and Skill Levels</td>
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<td>Erma Drobnis, PhD, HCLD (University of Missouri School of Medicine; Reproductive Medicine &amp; Fertility Solutions Inc.)</td>
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<td>9:15 a.m. - 9:35 a.m.</td>
<td>History of Sperm Morphology Classification Systems and Why They Have Failed; Introduction to Workshop Methods</td>
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<td>Susan A. Rothmann, PhD, HCLD (Fertility Solutions, Inc.)</td>
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<td>9:35 a.m. - 10:15 a.m.</td>
<td>Sperm Morphology Algorithm 101</td>
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<td>Anna-Marie Bort, MLT(ASCP)CME (Fertility Solutions, Inc.)</td>
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<td>10:15 a.m. - 10:30 a.m.</td>
<td>Break</td>
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<td>10:30 a.m. - 11:00 a.m.</td>
<td>Interactive Algorithm Instruction 1</td>
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<td>Anna-Marie Bort, MLT(ASCP)CME (Fertility Solutions, Inc.)</td>
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<td>11:00 a.m. - 12:00 p.m.</td>
<td>Practice 1 with Virtual Smears</td>
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<td>12:00 p.m. - 1:00 p.m.</td>
<td>&quot;LAB SCIENCE FORUM LUNCHEON &quot;Semen Analysis and Sperm Epigenetic Signatures: Diagnostic Potential&quot;</td>
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<td>Timothy Jenkins, PhD (University of Utah)</td>
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<td>Interactive Review of Virtual Smears</td>
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<td>1:45 p.m. - 2:15 p.m.</td>
<td>Interactive Instruction 2</td>
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<td>Anna-Marie Bort, MLT(ASCP)CME (Fertility Solutions, Inc.)</td>
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<td>2:15 p.m. - 2:45 p.m.</td>
<td>Practice 2 with Virtual Smears</td>
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<td>2:45 p.m. - 3:00 p.m.</td>
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<td>3:00 p.m. - 3:45 p.m.</td>
<td>Interactive Review of Virtual Smears</td>
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<td>3:45 p.m. - 4:05 p.m.</td>
<td>Round Cell Identification</td>
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<td>4:05 p.m. - 4:25 p.m.</td>
<td>Post Training Assessment</td>
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<td>4:25 p.m. - 4:40 p.m.</td>
<td>Application of the Algorithm in Modern Laboratories</td>
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<td>4:40 p.m. - 5:00 p.m.</td>
<td>Wrap-Up Discussion and Q&amp;A</td>
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SPEAKER ABSTRACTS

SATURDAY, APRIL 21, 2018
6:30 p.m. - 7:30 p.m.

EMIL STEINBERGER MEMORIAL LECTURE
Of Mice (Pigs, Monkeys) and Men: Animal Models to Study Germine Stem Cells and Spermatogenesis
Ina Dobrinski, Dr. med. vet., MVSc, PhD, Dipl. ACT
University of Calgary, Calgary, AB, Canada

Spermatogenesis is a process of cell proliferation and differentiation resulting in the continuous production of sperm throughout the adult life of the male. Supported by somatic cells, germ cells differentiate from diploid, undifferentiated spermatogonia to mature, haploid spermatozoa. To sustain this process, a constant supply of undifferentiated spermatogonia must be maintained by a pool of germline stem cells (GSCs). In the testis, GSCs reside in a unique microenvironment, the stem cell niche, which supports self-renewal and differentiation of GSCs. The niche is composed of Sertoli cells, adjacent differentiating germ cells, extracellular matrix, and interstitial components. The balance of self-renewal and differentiation of GSCs must be tightly regulated to maintain normal spermatogenesis, yet the mechanisms that govern this fate decision are largely unknown. Culture systems to expand and maintain germline stem cells from mice and rats are well-established in many labs, yet these systems are not readily translated to higher mammals including humans. Lack of appropriate tools to monitor GSC function, to generate sufficient numbers of GSCs for fertility preservation, and to develop alternatives to transplantation for screening of GSC function are identified as significant barriers in enhanced treatment of male infertility. Non-rodent animal models provide a platform to explore fundamental pathways and develop novel strategies to preserve and restore reproductive capacity in males. This will ultimately serve to increase the chances that couples may have biological offspring without using conventional assisted reproduction.

SUNDAY, APRIL 22, 2018
8:10 a.m. - 9:00 a.m.

SYMPOSIUM: ART and Andrology
WOMEN IN ANDROLOGY LECTURE: Restoring Predictive Value of Sperm Morphology
Susan Ann Rothmann, PhD, HCLD
Fertility Solutions, Inc.

Sperm morphology has historical value for predicting fertility outcomes has diminished in the last few decades. Ample data show that most labs using the Strict morphology scheme are overly critical with few normal sperm found even in fertile men. In many centers, morphology no longer correlates with success after assisted reproductive therapies. Many different interpretations of classification criteria exist as seen clearly in proficiency testing where intra-observer and inter-laboratory variation typically exceeds acceptable and useful limits. The WHO reference ranges for normal Strict morphology encompass median and upper limits that many laboratories never attain. The lack of a standardized method for the application of sperm classification criteria explains why so many different interpretations of the Strict classification scheme exist. In the 30 years of dissemination of the Strict scheme, subjective definitions of normal and borderline normal sperm largely replaced the original Strict definitions, making repeatability and training very difficult.

Our research to develop a more objective method included extensive review of photographs and definitions of normal, borderline and abnormal sperm from published papers and atlases. We obtained primary data by surveying classification of 155 sperm by 99 international experts. Using well-established principles of pathology and taxonomy classification, we developed a dichotomous key algorithm with 12 queries of sperm shape and size. Borderline normal forms are classified as a separate category using definitions described by Menkveld in 1990. 143 archived morphology smears were analyzed with the algorithm and compared to original values using subjective analysis. Strict normal median with the algorithm was 18%, compared to the original median of 4% (WHO 5th reference medians for unscreened men = 14%, fertile fathers = 15%). The algorithm was used to analyze 436 archived smears from the NIH Life Study and the distribution of values was similar to WHO 5th reference ranges. Regression analysis of 170 smears showed excellent inter-observer correlation (0.9). The method was also highly stable. An unexpected benefit was a reduced analysis time from 30 to 15 minutes. Because borderline sperm are classified independently, the algorithm can be used to determine percent normal forms for Traditional or Strict morphology schemes simultaneously or various indices. Participants at ASA Lab Workshops found the method was easy to use and adopt and typically became less critical using the algorithm. Supported by: NIH Grant R43 HD044383-01

SUNDAY, APRIL 22, 2018
9:00 a.m. - 9:40 a.m.

SYMPOSIUM: ART and Andrology
Characterizing Human SSCs for Transplantation
Brian P. Hermann, PhD
Department of Biology, The University of Texas at San Antonio; San Antonio, TX.

Spermatogenesis is a complex and dynamic cellular differentiation process critical to male reproduction and sustained by spermatogonial stem cells (SSCs). In 1994, Dr. Ralph Brinster and colleagues transplanted mouse SSCs into the seminiferous tubules of infertile recipient mice and observed donor spermatogenesis that was competent to produce progeny. SSC transplantation has since become the gold standard bioassay for experimental assessment of SSCs and may also have application to preserve and restore male fertility after iatrogenic insults. We previously established feasibility of this approach in rhesus macaques, providing the essential proof-of-principle for clinical translation. However, obtaining sufficient numbers of autologous SSCs for transplantation is a major barrier to successful application of SSC transplantation in the clinic and may require propagation (or even derivation) in vitro. Regardless of the source of putative human SSCs, though, validation of their phenotypic and functional characteristics prior to transplantation is warranted. A number recently published and ongoing studies have characterized populations of mouse spermatogonia highly enriched or depleted for SSCs in an effort to derive genome-wide gene expression and epigenome phenotypes correlated with stemness. To similarly characterize likely human SSCs, where definitive knowledge of SSC identity is absent due to lack of an assay, we recently catalogued single-cell transcriptomes for thousands of individual spermatogonia from immature and adult mice and adult humans. This allowed us to resolve human SSC and progenitor spermatogonia based on comparison to gene expression patterns in subpopulations of transplantation-validated mouse SSCs and progenitor spermatogonia. Investigation of human SSC epigenome features has similarly lagged, but recent studies using mixed populations of human spermatogonia provides insights into their likely characteristics. This talk will review published and unpublished results demonstrating the transcriptome and epigenome characteristics of mouse and human SSCs which provide essential benchmarks for comparison of cells intended for transplantation to restore male fertility.
Spermatogonial stem cell (SSC) loss due to cancer treatment, developmental disorder or genetic abnormality may cause permanent infertility. Cryopreservation of ejaculated sperm is an effective method of fertility preservation in adult males at risk of infertility. However, this is not an option in pre-pubertal boys because spermatogenesis has not yet started, and it is difficult in adolescents who are not sexually mature. Therefore, testicular tissue cryopreservation to preserve SSCs for future generation of spermatogenesis, either in vivo or in vitro, could be an option for these groups of patients. Although SSC transplantation has been successful in several species including non-human primates, it is still experimental in humans. There are several remaining concerns which need to be addressed before initiating trials of human SSC autotransplantation. Establishment of a testicular tissue banking system is a fundamental step towards using SSC technology as a fertility preservation method. It is important to understand the consultation, harvesting the testicular tissue, histological evaluation, cryopreservation, and long-term storage aspects. It is important to have a multidisciplinary approach to establish testicular tissue banking for males at risk of infertility.

**SYMPOSIUM: ART and Andrology**

**Human Spermatogonial Stem Cells (hSSCs) and Fertility Preservation – Lab Requirements and the Interface Between Clinic and Lab**

Hooman Sadri-Ardekani, MD, PhD
Assistant Professor of Urology & Regenerative Medicine, Director of Male Fertility Research Program, Wake Forest School of Medicine, Winston-Salem, NC

Currently, two strategies to restore fertility in-vivo after gonadotoxic treatments are being investigated: spermatogonial stem cell transplantation (SSCT) and testicular tissue grafting (TTG). TTG has the advantage to transplant spermatogonial stem cells (SSCs) within their own microenvironment. In animal models, this results in higher fertility restoration rates if performed intra-testicularly. However, TTG cannot be proposed to some former cancer patients because of the risk of re-introducing malignant cells. SSCT in combination with selective cell sorting could be a solution for these patients. However, so far, it remains questionable whether malignant cells can be effectively depleted from a testicular cell suspension, e.g. in leukemia. In the near future, TTG is implementable in the clinic for patients with no such risk. As this technique is still considered experimental, a clinical trial has to be set up and ethical approval should be sought. Patients fulfilling the inclusion criteria (>18y; child wish; azoosperma; no or low risk for presence of malignant cells in testis tissue) need careful counselling and information about the experimental nature of the procedure.

In order to maximize the success rate after transplantation, multiple fragments can be grafted on different locations (in testis, in scrotum). After transplantation, sperm and blood parameters have to be followed very closely. During the entire course of follow-up, psychological support should be provided. As we do not expect that sperm grown in transplanted tissue can reach the epididymis, TESE in combination with ART will probably be necessary. Since very little is known about the safety of TTG, also the children conceived with sperm obtained by this procedure should be followed. Ideally, transplantation and follow-up protocols for both transplanted men and their children are standardized and follow-up data are collected in an international registry.

**SUNDAY, APRIL 22, 2018**

9:55 a.m. - 10:30 a.m.

**SYMPOSIUM: Reconstructing Andrologic Function after Traumatic Injury to Brain and Body**

**AUA LECTURE: Genital Blast Injuries: Andrologic Consequences and Therapeutic Conundrums**

Robert C. Dean, MD
Walter Reed National Military Medical Center

Combat actions for Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) have resulted in significant pelvic and gentle injuries for combat warriors. Unlike any other major military actions, the casualty survival rate is the highest in military history for these conflicts. Casualty survivability has reached almost 90%. This is a significant improvement from Vietnam combat action with the casualty survival ability rate of 76%. With this increased survivability, rehabilitation care has been pushed to develop new methods of treatment in an attempt to return normalcy to these severely injured warriors. The combat actions in Iraq and Afghanistan had produced better understandings of traumatic brain injury, extremity wounds, and genitourinary injuries. Historically genitourinary injuries and combat situations occur on average of 2-5%. Due to the increase in casualty survivability, the genitourinary injury rate for operations OEF and OIF is approximately 10%. With this increase genitourinary injury rate treatment plans to preserve hormonal function and fertility have started to be investigated. In an evaluation of 136 patients with testicular injury since the onset of combat actions, the need for testosterone supplementation is necessary in more severe injury as compared to minor injury, as expected. In men who suffer combat-related trauma without genitourinary injuries the average time for return of normal testosterone levels is approximately 9 months. The use of testosterone supplementation in men with significant genitourinary injuries is generally initiated within 2-3 months from date of injury. Unfortunately, infertility rates are quite high among any patient with genitourinary injury caused from combat wounds. New surgical techniques and prevention models have been developed to aid with fertility preservation. Seminal Vesicle sperm harvest has been employed for sperm preservation. 6 cases of sperm harvest from the seminal vesicle will be discussed with outcomes data. Presently the military is developing research investigations for the preservation of testis tissue and fertility.

**SYMPOSIUM: Reconstructing Andrologic Function after Traumatic Injury to Brain and Body**

**The Trauma Outcomes and Urogenital Health (TOUGH) Project: Initial Findings and Future Directions**

Steven J. Hudak, MD
Brooke Army Medical Center, San Antonio, TX

Improvements in combat casualty care during recent conflicts in Iraq and Afghanistan led to unprecedented rates of survival despite a rise in overall injury severity among U.S. service members (SMs) wounded in these conflicts. As a direct consequence, many U.S. SMs have faced the...
exhaustive process of recovery, rehabilitation, and functional restoration following complex orthopedic, gastrointestinal, and genitourinary (GU) injuries which in previous conflicts would likely have been unsurvivable. The unique impact of the sensitive and intimate nature of GU injuries on the process of recovery after combat injuries has received little attention in previous conflicts. Thus, the Trauma Outcomes and Urogenital Health (TOUGH) Project was designed to evaluate the long term sexual, urinary, reproductive, and psychological outcomes among the large number of U.S. SMs who sustained GU injury during Operation Iraqi Freedom and Operation Enduring Freedom (OIF/OEF). During the first phase of the TOUGH Project, a query of the Department of Defense Trauma Registry identified 1,462 SMs who sustained one or more GU injuries. Genital injuries predominated and comorbid extremity amputation(s) and colorectal injuries were common. Ongoing prospective evaluation of the TOUGH cohort will hopefully provide much needed information on the potential long-term sequelae of battlefield GU injury.

**SUNDAY, APRIL 22, 2018**

3:55 p.m. - 4:30 p.m.

**SYMPOSIUM: Reconstituting Andrologic Function after Traumatic Injury to Brain and Body**

**Neurobiology of Traumatic Brain Injury: Hypogonadism, Biomarkers and Implications for Treatment and Recovery**

Amy K. Wagner, MD
University of Pittsburgh

**Introduction:** Post-traumatic hypopituitarism is a prevalent complication of traumatic brain injury (TBI). Hypogonadotropic hypogonadism is one of the most common post-traumatic hypopituitarism deficiencies, and it is associated with poor outcomes after TBI. The pathogenesis of persistent hypogonadotropic hypogonadism (PHH) remains unclear, although autoimmune and inflammatory mechanisms have been proposed to contribute to this process.

**Methods:** We conducted a prospective longitudinal cohort study of men with severe TBI (n=61) recruited from a university hospital level 1 trauma center and compared them to 63 healthy men. We measured serum testosterone and luteinizing hormone levels as well as levels of IgM and IgG autoantibodies against pituitary (APA) and hypothalamus (AHA) via a custom developed ELISA from 2-26 weeks post-TBI. Tissue specificity of the APA and AHA was confirmed using fluorescence immunohistochemistry with human pituitary and hypothalamus sections, respectively. We then compared autoantibody levels among those with and without PHH. The impact of aging on PHH status, as well as autoantibody levels, were assessed using both regression and mediation analyses. Clinical prediction of PHH status using autoantibodies, LH, and testosterone was explored using receiver operating curve analysis

**Results:** The PHH group median age was 11 years older than the non-PHH group (35 vs. 24 years, p=0.02). There were no significant differences between PHH vs. non-PHH groups in body mass index, education level, race, GCS score, injury severity score, length of hospital stay, or mechanism of injury. Of men with TBI, 24 (39%) were injured at work. We then compared autoantibody levels among those with and without PHH. The impact of aging on PHH status, as well as autoantibody levels, were assessed using both regression and mediation analyses. Clinical prediction of PHH status using autoantibodies, LH, and testosterone was explored using receiver operating curve analysis

**Conclusion:** There is a relationship between higher APA IgM levels and the absence of PHH after severe TBI, which may suggest a role for protective autoimmunity against hypogonadism development in the first 6 months post-TBI. Testosterone, age, and auto-antibody profiles may be useful for clinical delineation of PHH.

**Funding:** NIDILRR 90DP0041, CDC grant # R49/CCR323155, DODW81XWH-071-0701, UPMC Rehabilitation Institute, and UPP Foundation.

**SUNDAY, APRIL 22, 2018**

4:30 p.m. - 5:05 p.m.

**SYMPOSIUM: Reconstituting Andrologic Function after Traumatic Injury to Brain and Body**

**Spermatogonial Stem Cells to Preserve and Restore Fertility**

Kyle Orwig, PhD
Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine

Chemotherapy or radiation treatments for cancer or other conditions can cause permanent infertility. Adult men have the option to cryopreserve a semen sample before treatment, which can be used in the future to achieve a pregnancy using established assisted reproductive technologies. This option is not available to prepubertal boys who are not yet producing sperm. This is an important human health concern because most boys will survive their cancer and have their entire reproductive life in front of them. Each year in the United States, there are nearly 2,500 survivors of childhood cancers or bone marrow transplantation who will be at risk of permanent infertility due to their treatment. With this in mind, centers in the US and abroad have been freezing testicular tissues for “at risk” patients with anticipation that spermatogonial stem cells in those tissue can be used in the future to produce fertilization-competent sperm. The Fertility Preservation Program in Pittsburgh and Coordinated Centers (https://fertilitypreservationpittsburgh.org/) have frozen testicular tissues for 130 male patients since 2011. This lecture will review stem cell therapies that are currently in the research pipeline and their prospects for translation to the human fertility clinic.

We are grateful to the Scaife Foundation, the Richard King Mellon Foundation, the Magee-Womens Research Institute and Foundation and the University of Pittsburgh departments of Ob/Gyn & Reproductive Sciences and Urology who have generously supported the Fertility Preservation Program in Pittsburgh. It is in this context that we have had the opportunity to meet the infertile patients that fuel our passion for fertility research. Research in the Orwig laboratory is supported by the Eunice Kennedy Shriver National Institute for Child Health and Human Development (HD055475, HD076412, HD075795, HD092084, HD061289), the US-Israel Binational Science Foundation and gift funds from Montana State University and Sylvia Bernassoli.
Endocrine Disruption Associated with Developmental Exposure to a Mixture of Hydraulic Fracturing (Fracking) Chemicals

Susan C. Nagel1, Victoria Balise1, Christopher D. Kassotis1,2, Jennifer Cornelius Green1, Erma Drobni3, and Tim Evans3
1Department of Obstetrics Gynecology and Women’s Health, University of Missouri, 2Nicholas School of the Environment, Duke University, 3Veterinary Diagnostic Laboratory, University of Missouri

Unconventional oil and gas (UOG) extraction combines directional drilling with hydraulic fracturing “fracking” to release oil and natural gas from previously inaccessible reserves. Fracking involves the injection of millions of gallons of water, chemicals and sand under high pressure to fracture underground rock. Billions of gallons of wastewater containing fracturing and naturally occurring chemicals are produced each year. We measured the endocrine disrupting potential of 24 UOG chemicals and found antagonism for one or more of the estrogen, androgen, glucocorticoid, thyroid hormone, and progesterone receptors for 23 chemicals. An equimass mixture of these chemicals showed less than additive activity for androgen and glucocorticoid receptors, additive activity for the progesterone and estrogen receptors and more than additive activity for the thyroid hormone receptor. We assessed adult outcomes following developmental exposure via maternal drinking water to a mixture of 23 of these chemicals at four doses in two different exposure windows in C57Bl6 mice. We found overlapping and distinct outcomes in male mice following a prenatal (gestation day 11 to 19) versus a combined prenatal and postnatal exposure window (gestation day 1 to postnatal day 21). Body weight was increased in male weanlings after prenatal exposure and decreased with combined prenatal and postnatal exposure, however body weight changes did not persist into adulthood. Male offspring with prenatal exposure had reduced caudal sperm counts at 30 and 300 µg/kg mixture per day, increased testes weights at 3 and 3000 µg/kg and altered serum testosterone concentrations at 300 and 3000 µg/kg. On the contrary, male offspring with combined exposure had increased caudal sperm and decreased apoptosis at 150 µg/kg per day, increased motility at 1.5, 15 and 150 µg/kg per day, and decreased testes weight at 15 µg/kg per day. Preliminary analyses suggest that exposure during the combined window adversely affected seminiferous tubules and the cycle of the seminiferous epithelium in male offspring at 3 months of age. These and other ongoing studies are aimed at identifying the underlying mechanisms for these effects. Taken together, these data suggest that developmental exposure to UOG chemicals at potential environmentally relevant levels may have negative impacts on male reproductive endpoints in exposed animals. Future studies should examine whether UOG exposure is associated with negative reproductive health outcomes in humans.
Zika virus is now recognized as a cause of congenital neurologic birth defects, notably microcephaly, and has been associated with potentially fatal complications such as severe thrombocytopenia and Guillain-Barre syndrome. Although most Zika infections are transmitted by infected mosquitoes, Zika virus transmission has been documented through sexual contact, blood transfusion, laboratory exposure, and both intrauterine and intrapartum transmission. Zika virus RNA has been detected in semen, urine, saliva, cerebrospinal fluid, vaginal or cervical secretions, and other body fluids. Most transmissions through sexual contact have been from men with symptomatic infection to their female partners. However, sexual transmission has also occurred from asymptomatic men, through male-to-male and female-to-male sex, and possibly through oral sex. Shedding in the female genital tract appears to be of short duration. In contrast, there are reports of prolonged detection of ZIKV RNA in semen, with the longest reported duration of detection up to 370 days after onset. The localization of Zika virus in the human genital tract and its consequences are not fully known. However, studies in monkeys and mice have evidenced Zika virus in different male genital organs and have shown the negative effects of Zika on the testis and epididymis. Prospective studies in humans have shown a transient decrease in sperm count and multiple sperm anomalies among men with Zika virus RNA-positive semen specimens. We have been studying the frequency and duration of detectable Zika virus RNA in human body fluids, by prospectively assessing a cohort of newly infected patients in Puerto Rico. As part of this study, we have also evaluated the individual, sexual, and household factors associated with ZIKV prevalence among household contacts of symptomatic persons who presented to care with viremic ZIKV infection. In this presentation, I will summarize the public health implications of Zika virus infections among household contacts of symptomatic persons who presented to care with viremic ZIKV infection. I will also discuss what we know about the risk of sexual transmission, and review the current recommendations to prevent Zika virus sexual transmission.
Organic compound, EP055, which targets EPPIN on the surface of human spermatozoa and Eppin Pharma has developed a small molecule to inhibit human sperm motility. Therefore, we have searched for small organic compounds that would substitute for SEMG1 or a-EAb and cause a loss of sperm function. EP055 was tested in cynomolgus (Macaca fascicularis) males to determine its plasma half-life after intravenous (i.v.) infusion of a single dose and for binding to its target tissues. Examination of macaque testis, epididymis, and plasma after i.v. infusion of a single dose of compound demonstrated that EP055 was detected in testis and epididymis two hours and six hours post-infusion. We initiated a trial in rhesus (Macaca mulatta) males to assess the availability of EP055 in semen and its effect on sperm motility as a measure of the drug’s efficacy. Four macaques were infused with a low dose followed by a recovery period and a subsequent high dose of EP055. After high dose administration no normal sperm motility was observed at 30 hours post-infusion. Recovery of sperm motility was observed by 78 hours post-infusion; with full recovery in all animals by 18 days post-infusion. EP055 has the potential to be a male contraceptive that would provide a reversible, short-lived pharmaceutical alternative.

**TUESDAY, APRIL 24, 2018**

1:55 p.m. - 2:50 p.m.

**SYMPOSIUM: Male Contraception**

**Current Paradigms in Contraception Method Development**

Daniel S. Johnston, PhD

NIH, Contraception Research Branch NICHD

Nearly half of all pregnancies in the United States are unintended. There is a critical need for fertility regulation methods that fit the needs of women and men throughout their reproductive lives. The Contraception Research Branch (CRB) within the Eunice Kennedy Shriver National Institute of Child Health and Human Development supports basic, applied, behavioral, translational, and clinical research for the development of new and improved male and female methods of contraception. The goal of the presentation will be to (1) review the product development process, (2) outline the benefits and challenges of current and emerging contraceptive strategies, (3) present research from selected ongoing contraceptive programs, and (4) discuss current funding opportunities in contraception method development.

2:50 p.m. - 3:20 p.m.

**SYMPOSIUM: Male Contraception**

**DEVELOPMENT OF A NEW NONHORMONAL MALE CONTRACEPTIVE DRUG: UPDATE**

Michael G. O’Rand, PhD

Eppin Pharma Inc, Chapel Hill, NC; University of North Carolina at Chapel Hill, Chapel Hill, NC

Men have two practical choices for contraception; the condom which has a high typical use failure rate or vasectomy. A new male non-hormonal contraceptive that targets the motility and delivery of sperm has been developed by Eppin Pharma Inc. EPPIN is a male-specific protein found on the surface of human spermatozoa and Eppin Pharma has developed a small organic compound, EP055, which targets EPPIN on the surface of human sperm and inhibits motility. Based on previous work establishing that EPPIN is essential for fertility in primates we searched for small organic compounds to substitute for semenogelin (SEMG1) or anti-EPPIN antibodies (a-EAb), both of which inhibit sperm motility and have overlapping binding sites on the C-terminal domain of EPPIN. In order to optimize our lead compounds for binding to the C-terminal domain of EPPIN, we first characterized SEMG1’s binding to EPPIN and defined the minimal sequence necessary to inhibit human sperm motility. Therefore, we have searched for small organic compounds that would substitute for SEMG1 or a-EAb and cause a loss of sperm function. EP055 was tested in cynomolgus (Macaca fascicularis) males to determine its plasma half-life after intravenous (i.v.) infusion of a single dose and for binding to its target tissues. Examination of macaque testis, epididymis, and plasma after i.v. infusion of a single dose of compound demonstrated that EP055 was detected in testis and epididymis two hours and six hours post-infusion. We initiated a trial in rhesus (Macaca mulatta) males to assess the availability of EP055 in semen and its effect on sperm motility as a measure of the drug’s efficacy. Four macaques were infused with a low dose followed by a recovery period and a subsequent high dose of EP055. After high dose administration no normal sperm motility was observed at 30 hours post-infusion. Recovery of sperm motility was observed by 78 hours post-infusion; with full recovery in all animals by 18 days post-infusion. EP055 has the potential to be a male contraceptive that would provide a reversible, short-lived pharmaceutical alternative.
SYMPOSIUM: Male Contraception
Past, Present, and Future of Vas-Occlusive Medical Devices
Kevin Eisenfrats, BS
Co-Founder and CEO of Contraline, Inc.

Vas-occlusion, a concept initially introduced the late 1960s, describes a method for inducing infertility in the male by implanting a device into the vas deferens to block sperm transport. In the past five decades, there have been many attempts at achieving vas-occlusive contraception using formed-in-place plugs and in situ forming materials. Yet, to date, no products have successfully gained regulatory approval or reached the stages of commercialization. Hydrogels are materials that can be applied to the field of vas occlusion given their unique chemistry and biological properties. Efforts in the United States are underway to develop novel forms of hydrogels that are both long lasting and reversible. The development of vas-occlusive medical devices poses great challenges, and if overcome, the future holds significant opportunity to advance the field of male contraception. Contraline will be highlighted as one such company working through the medical device development process towards FDA approval.
1 CRISPR-CAS9 MEDIATED KNOCKDOWN OF ODF2 IN PORCINE TESTICULAR SOMATIC CELLS
Taylor Goldsmith BSc, Dennis Webster¹, Dan Carlson PhD² and Ina Dobrinski DVM, PhD³ ¹Recombinetics Inc; ²University of Calgary
(Presented By: Taylor M. Goldsmith, BSc)

Most of the cells in the body possess non-motile primary cilia. They can act as sensors, mediate cell-to-cell communication, and are essential in development and for differentiation of stem cells. Primary cilia are present on somatic cells of the testis, with the number of cilia decreasing as the testis matures. This suggests that primary cilia are important in the morphogenesis of the testes. We hypothesize that ablation of primary cilia from testicular somatic cells will inhibit testicular morphogenesis. To test this, we targeted outer dense fiber protein 2 (ODF2), a protein essential for cilia formation, using CRISPR-Cas9 in porcine testicular somatic cells. We then investigated the effects of primary cilia ablation on tubular morphogenesis in vitro. Testicular somatic cells were isolated from one week old piglets through enzymatic digestion and separated from germ cells by differential plating. Two exons of Odf2 were targeted using the CRISPR-Cas9 system with Odf2 guide RNA delivered using nucleofection. Cilia loss was assessed by immunofluorescence for ARL13B, a ciliary GTPase. Cells transfected with a CRISPR construct lacking the guide RNA sequence served as controls. To study tubular morphogenesis in vitro, cells were cultured on Matrigel and analyzed for tubule formation by immunofluorescence. Treatment with CRISPR-Cas9 caused a significant reduction in the number of primary cilia on testicular somatic cells (p=0.0001). In targeted cells, 23.8% +/- 6.26 of cells had cilia, compared to 68.74% +/- 6.26 unmodified cells with cilia (n=4). Quantitative PCR confirmed knockdown of Odf2 expression in cells that were transfected with the CRISPR constructs, with 5.6-fold lower expression of Odf2 compared to controls (n=2). When transfected cells were used to form tubules in vitro, cells where Odf2 had been targeted formed thinner, shorter, and less organized tubules compared to tubules formed from sham edited controls. These results indicate that primary cilia play a role in tubular morphogenesis in vitro and this approach will provide the basis for elucidating signaling pathways involved in tubule formation. Supported by NIH/ORIP R01 OD016575-12.

2 INVESTIGATING ZIKA VIRUS PATHOGENESIS ON MALE REPRODUCTION USING A HUMAN 3-DIMENSIONAL TESTICULAR ORGANOID MODEL
Nima Pourhabibi Zarandi MD¹, Daniel Strange², Goral Trivedi³, Anthony Atala MD⁴, Colin Bishop PhD⁵, Saguna Verma PhD⁶ and Hooman Sadri-Ardekani MD, PhD⁷ ¹Wake Forest Institute for Regenerative Medicine; ²Recombinetics Inc; ³University of Calgary, ⁴Wake Institute for Regenerative Medicine; ⁵University of Hawaii at Manoa, ⁶Wake Forest Institute for Regenerative Medicine; ⁷University of Calgary
(Presented By: Nima P. Zarandi, MD)

Introduction: An increase in the number of newborns with microcephaly in women infected with Zika virus (ZIKV) in the 2015-2016 global epidemic coincided with the detection of ZIKV in the semen. This raised concerns regarding the sexual transmission of this primarly mosquito-transmitted virus. Recent reports of detecting ZIKV in seminal fluid months after clearance of the viremia in as high as 56% of infected men, together with a low sperm count, suggested the ability of ZIKV to enter into the immune privileged compartment of the testes and establish persistent infection. The lack of any specific therapeutic drugs to clear testicular infection and the absence of any appropriate animal model to mimic human testicular ZIKV infection has brought an urgent need for the development of human in vitro systems to understand the associated mechanisms of viral persistence.

Objective: Utilize a 3D human testicular organoid system to support robust infection of ZIKV as a novel model system to assess the cell tropism of the virus and infected testicular cell survival.

Methods: Cryopreserved, multicellular 3D human testis organoids (HTOs) constructed from cultured adult human testicular cells were thawed and used after a 5-day recovery period. HTOs were infected with 105 PFU of ZIKV (PRVABC59) for 1 hour at 37°C. Twenty four and 72 hours after infection RNA virus copy number, plaque assay, ATP activity and cells specific gene expression were compared between control and infected HTOs.

Results: Low copies of ZIKV RNA were detected at 24hrs that increased significantly by more than 2 logs at 72 hours. A similar trend was observed in the plaque assay with a significant increase in infectious virions at the 72hr time point. ATP production showed a dramatic decrease after 72hrs that is comparable with the peak virus copy numbers. Expression levels of ZBTB16 (Spermatogonial cell marker), CYP19A1 (Sertoli Cell marker) and STAR (Leydig cell marker) were down regulated significantly after 72 hours.

Conclusion: This preliminary study provides evidence that ZIKV can efficiently infect HTOs and affect different types of testicular cells. Future research using this 3D HTO system will focus on long-term replication and persistence of ZIKV in the testes. This may open exciting new avenues for basic and translational research on testes-tropic viruses including testing efficacy and toxicity of anti-virus drugs in clearing testicular infection.

3 IN VITRO CULTURE OF HUMAN KLINEFELTER SPERMATOGONIAL STEM CELLS
Guillermo Galdon MD¹, Nima Pourhabibi-Zarandi MD¹, Mark Pettenati Ph.D², Stuart Howards MD³,⁴, Stanley Kogan MD FACS³,⁴, Anthony Atala MD³,⁴ and Hooman Sadri-Ardekani MD PhD³,⁵ ¹Wake Forest Institute for Regenerative Medicine; ²Section of Medical Genetics, Department of Pathology Wake Forest School of Medicine; ³Department of Urology; ⁴Wake Forest School of Medicine; ⁵Wake Forest Institute for Regenerative Medicine
(Presented By: Guillermo G. Lopez, MD)

Introduction: Klinefelter Syndrome (KS) is characterized by masculine phenotype, supernumerary sex chromosomes (47, XXY) and impaired fertility due to progressive loss of spermatogonial stem cells (SSC) starting at the onset of puberty. It’s been hypothesized that early SSC cryopreservation could be an option for future fertility treatments in these patients including SSC transplantation. Establishment of an in vitro culture system is critical to preserve future fertility in KS patients.

Objectives: Isolate and propagate human KS spermatogonia stem cell from cryopreserved tissue.

Methods: Testicular tissue from KS patients enrolled in experimental testicular tissue bank at Wake Forest Baptist Health has been cryopreserved. Testicular cells were isolated and propagated in long term culture. Propagated testicular cells were characterized using q-RTPCR, digital-RT PCR, Flow Cytometry and MACS-sorted X/Y chromosomes FISH staining.

Results: Growing KS human testicular cells from 3 non-mosaic KS patients (13, 15 and 17 years old) showed viable testicular cells up to 120 days in culture. During this period, cells were expanded more than 2-million-fold. Cell specific gene expression revealed the presence of all 4 testicular cell types including Spermatogonia, Sertoli, Leydig and Peritubular cells. A population of ZBTB16+ undifferentiated spermatogonia (25% of all cultured cells) was identified all along culture using digital PCR. Flow Cytometry analysis detected a HLA- /CD9+/CD49f+ population (8% of all cultured cells) suggesting a stem cell subpopulation. FISH staining for chromosomes X and Y on CD9+ MACS sorted spermatogonia confirmed KS sexual trisomy along the culture. Interestingly, using serial FISH detected a population of cells losing the extra X chromosomes in later passages (Figure 1).
Conclusions To our best knowledge this is the first report of successful isolation and propagation of testicular cells from human KS patients. This culture system has potential for either in vitro or in vivo fertility preservation in KS patients.

4 PREGNANCY ESTABLISHED AFTER AUTOLOGOUS GRAFTING OF CRYOPRESERVED TESTICULAR TISSUE FROM PREPUBERTAL RHESUS MACAQUES

Adetunji Fayomi DVM, MVSc1, Peters Karen BS2, Meena Sukhwani PhD3, Cathy Ramsey BS3, Carol Hanna PhD3, Jon Hennebold PhD4 and Kyle Orwig PhD5 1Magee-Womens Research Institute, University of Pittsburgh School of Medicine; 2Magee-Womens Resarch Institute University of Pittsburgh School of Medicine, Pittsburgh, PA; 3Assisted Reproductive Technology Core, Oregon National Primate Research Center, Beaverton, OR  

Introduction and Objective: Testicular tissue freezing is an experimental fertility preservation option for prepubertal boys who are not able to cryopreserve sperm. The objective of this study was to demonstrate that fertilization competent sperm could be recovered from frozen/thawed grafts from prepubertal Rhesus macaques.

Materials and Methods: Testicular tissue of five prepubertal Rhesus macaques was collected and cut into small pieces (2-5mm3). Fresh or frozen/thawed tissues were grafted under the back skin (3 fresh & 3 frozen) and in the scrotum (1 fresh & 1 frozen). Matrigel was introduced into four sites on the back and both scrotal sites. Grafts were recovered for analysis after 8-10 months. Graft volume, follicle stimulating hormone (FSH) and testosterone (T) levels increased after grafting, indicating progression through puberty and an intact hypothalamic, pituitary, gonadal axis. Graft weights from the scrotum were higher than from the back (P<0.01), but complete spermatogenesis was confirmed in all grafts with spermatids in more than 70% of seminiferous tubules. Irrespective of experimental group, millions of sperm were recovered per graft and sperm were competent to fertilize Rhesus oocytes, leading to preimplantation embryo development up to the hatching blastocyst stage, in vitro. Embryos were transferred into surrogate Rhesus females which results in pregnancy. Evaluation of fetal development is ongoing.

Results: HE and IF confirmed that donor tissues were immature (no spermatogenesis) at the time of grafting. Irrespective of the graft site or matrigel treatment, graft volume increased over time in the frozen/thawed as well as fresh grafts. FSH and T levels increased after grafting, indicating progression through puberty and an intact hypothalamic, pituitary, gonadal axis. Graft weights from the scrotum were higher than from the back (P<0.01), but complete spermatogenesis was confirmed in all grafts with spermatids in more than 70% of seminiferous tubules. Irrespective of experimental group, millions of sperm were recovered per graft and sperm were competent to fertilize Rhesus oocytes, leading to preimplantation embryo development up to the hatching blastocyst stage, in vitro. Embryos were transferred into surrogate Rhesus females which results in pregnancy. Evaluation of fetal development is ongoing.

Conclusion: Testicular tissue grafting is a mature technology that may be ready for the human fertility clinic. Support: This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development grant HD075795.

5 PUBERTAL DEVELOPMENT AFTER TESTICULAR TISSUE BIOPSY FOR FERTILITY PRESERVATION.

Aude Brayé PhD-student1, Inge Gies Prof Dr², Veerle Vloeyberghs Dr³, Alina Ferster Prof Dr³, Jutte Van der Werff Ten Bosch Prof Dr², Herman Tournaye Prof Dr² and Ellen Goossens Prof Dr³ 1Vrije Universiteit Brussel; 2Université Libre de Bruxelles; 3Hôpital Universitaire Des Enfants Reine Fabiola  

Introduction: Male infertility and the search for an adequate fertility preservation strategy becomes clinically more relevant because of the improved survival rate of childhood cancer patients and the earlier diagnosis of Klinefelter Syndrome (KS) patients due to better diagnostic methods. Currently, the only fertility preservation option for these young boys is undergoing a testicular tissue biopsy followed by testicular tissue banking. Although recent evidence demonstrates that the immediate adverse effects of the biopsy procedure are rare (1%), nothing is known on its possible adverse effects on the long-term. The aim of this project is to investigate how a testicular tissue biopsy procedure at young age may affect the pubertal development of boys.

Methods: All paediatric patients needing gonadotoxic treatment or patients with KS seen at the Universitair Ziekenhuis Brussel between 2002 and 2016 were enrolled in this cohort study. Hormone serum levels important during pubertal development, testicular volume, bone age and bone density were recorded at different time points over several years and compared between patients who underwent a testicular tissue biopsy and those who did not. Data were analysed using the Chi-squared statistical test.

Results: 113 boys needing gonadotoxic treatment and 109 KS patients were enrolled in this retrospective cohort. The testes of 63 boys needing gonadotoxic treatment (56%) and the testes of 37 KS patients (34%) were biopsied. No differences were observed for luteinizing hormone, follicle-stimulating hormone, testosterone, inhibin-B, testicular volume and bone age between patients who did and those who did not undergo a testicular tissue biopsy. For anti-müllerian hormone, estradiol and bone density, not enough data were available to make any conclusions. We also observed that the need for substitution treatment occurred at the same Tanner stage for patients who did and those who did not undergo a testicular tissue biopsy.

Conclusion: These results demonstrate that a testicular tissue biopsy at young age has no additional adverse effect on the pubertal development of young boys. The large number of missing data proves the need to establish a more standardized follow-up protocol for boys undergoing testicular tissue biopsy at young age.
In mammals, initiation of motility and acquisition of the ability to fertilize eggs develop during passage of sperm through the epididymis. The mechanistic details of the biochemical basis for epididymal sperm maturation are not known. Changes in cAMP action and protein phosphorylation were thought to be involved. Two new discoveries should enable us to understand the biochemical basis underlying epididymal sperm maturation. One of the signaling proteins identified by gene deletion to play a role in epididymal sperm maturation is the calcium regulated protein phosphatase calcineurin. Another signaling protein is glycogen synthase kinase 3 (GSK3). We identified GSK3 as a regulator of the sperm-specific protein phosphatase. The only phenotype of mice with global or selective knock out of Gsk3a in developing spermatocytes/spermatids is male infertility. Caudal epididymal sperm lacking GSK3a resemble wild type immature caput epididymal sperm. The purpose of this study was to examine the interrelationships between calcineurin, PP1g2, and GSK3a in acquisition of motility and fertilizing ability during their passage through the epididymis. We show that both calcineurin and PP1g2 affect GSK3a activity. We have verified earlier data that immature epididymal sperm contain significantly higher calcium levels compared to mature caudal epididymal sperm suggesting that an active calcineurin is essential during sperm maturation. We determined that the association of the regulators of the catalytic activity of PP1g2, inhibitors I2 and I3 and sds22, change during epididymal sperm maturation. Changes in the association of the regulators with PP1g2 are impaired in sperm lacking GSK3a resulting in reduced ATP generation through glycolysis. Surprisingly, sperm from calcineurin knock out mice have higher ATP levels compared to wild type sperm. Lack of calcineurin leads to an abnormal increase in mitochondrial potential and ATP production that is not synchronized with development of sperm motility and fertility potential. Sperm lacking GSK3 or calcineurin are also unable to fertilize eggs in vitro. Wild type sperm treated with calcineurin or GSK3 inhibitors reduce in vitro fertilization of eggs. Taken together our data show that calcineurin and GSK3a are the two missing pieces essential to the understanding the biochemical basis underlying activation of metabolism that accompanies sperm motility and fertility development in the epididymis. Supported by (NIH HD086839).

The golgin GMAP-210, also known as Thyroid Hormone Receptor Interactor 11 (TRIP11), is a cis-Golgi network-associated protein. The protein is required for efficient trafficking in the secretory pathway. Mice lacking GMAP-210 die at birth with a pleiotropic phenotype that includes growth restriction, ventriculoseptal defects of the heart, omphalocele, and lung hypoplasia. It has been shown that GMAP-210 determines IFT20 subunit of intracellular transport complex localization in the Golgi membranes lacking GMAP210, the Golgi structure, but IFT20 is no longer localized to this organelle. Our laboratory discovered that IFT20 is essential for male fertility and spermiogenesis in mice. To investigate the role of GMAP-210 in male fertility and spermatogenesis, the floxed Gmap-210 mice were bred with Stra8−/−cre mice so that the Gmap-210 gene is disrupted in spermatocytes/spermatids. The Gmap-210;Stra8−/−cre mutant mice did not show any gross abnormalities, and all of the mutant mice survive to adulthood. There was no difference in testis weight/body weight between controls and mutant mice. Histological examination of the testes revealed normal seminiferous tubule structure. Sperm number was slightly reduced compared to the controls. All adult homozygous mutant males examined had significantly reduced sperm motility. Sperm flagella appear to be normal as examined by light microscopy; however, almost all the heads were abnormally shaped. In the control mice, IFT20 was present in the Golgi bodies of spermatocytes, the acrosome, and manchette of spermatids. In the conditional Gmap-210 knockout mice, IFT20 was not present in these localizations; instead, IFT20 signal was scattered throughout the cytoplasm. An acrosome maker, peanut-lectin was almost absent in the spermatids of the conditional Gmap-210 knockout mice. Collectively, our findings suggest that GMAP-210 is essential for acrosome formation and sperm motility. Even though GMAP-210 determines IFT20 localization in male germ cells, and IFT20 is required for normal spermiogenesis and sperm flagella formation, other mechanisms might compensate for normal sperm flagella formation in the absence of GMAP-210 in male germ cells.
NEW INSIGHTS INTO THE VITAMIN D CONCENTRATION AND ITS ASSOCIATION WITH SEMEN QUALITY IN MALE SUBJECTS

Inari Ciccone MSc Student¹, Juliana Pariz MD, PH², Elaine Costa MD, PH², Parviz Gharagozloo MD, PH², John Aitken PHD⁴ and Jorge Hallak PHD⁴ ¹Department of Urology, University of Sao Paulo. Brazil/ Androscience -- High Complexity Clinical and Research Andrology Laboratory. Brazil; ²Department of Endocrinology, University of Sao Paulo. Brazil/ Androscience -- High Complexity Clinical and Research Andrology Laboratory. Brazil; ³CellOxess LLC; ⁴CellOxess LLC/ University of New Castle, Callaghan, NSW Australia; ⁵Department of Urology. University of Sao Paulo. Brazil/ Androscience -- High Complexity Clinical and Research Andrology Laboratory. Brazil/ CellOxess LLC

(Presented By: Inari Ciccone, Msc Student)

Background: Vitamin D is a versatile signaling molecule, that targets also male reproductive organs, in addition to the classic effects on bone, calcium and phosphate homeostasis. Accumulating evidences from animal and human studies suggest that vitamin D is involved in reproduction functions in both genders. Objective: To evaluate the vitamin D relationship with semen quality in male with seminal parameters alteration and normozoospermic diagnosis.

Patients and Methods: We selected 260 men (aged 18 to 60 y.o.) from a private andrology reference medical clinic. They were divided in two groups: Group 1: Normal seminal parameters (n=124) and Group 2: Abnormal seminal parameters (n=136). 25(OH) vitamin D serum concentration and lifestyle data were collected. Semen was analyzed according to WHO 2010 guidelines, PH, volume, motility, concentration, morphology, strict criteria and sperm functional tests were performed (ROS, CK, beads). Additionally, karyotype, frequency of varicocele, smoking, alcohol ingestion, and body composition were considered. Statistical analysis was performed by SPSS program version 19.0 (SPSS Inc., Chicago, IL). Spearman correlation, Mann-Whitney test and regression model were applied. Statistical significance was considered with P value < 0.05.

Results: The mean 25(OH)D concentration were significantly lower in Group 2 (p=0.016) and all seminal parameters had a positive correlation with 25(OH)D serum levels. The highest correlation coefficient value was observed on the association of total motility with Vitamin D (p=0.001). No significance influence was observed among the lifestyle data, body composition and varicocele with seminal parameters. All patients had normal karyotype.

Conclusion: Our results demonstrated that 25(OH)D levels has a positive influence on spermatogenesis and semen quality, suggesting that vitamin D replacement should highly be considered on male fertility treatment once low vitamin D levels have reached epidemic proportions in industrialized cities like Sao Paulo.

MALIGNANCY NEGATIVELY ASSOCIATED WITH BASELINE SEMEN PARAMETERS AMONG SPERM BANKERS

Rena Xu MD, MBA¹, Grace Centola PhD² and Cigdem Tanrikut MD³ ¹Massachusetts General Hospital; ²New England Cryogenic Center

(Presented By: Rena Xu, MD, MBA)

Introduction and Objective: Whether various forms of malignancy are associated with diminished semen quality prior to spermatotoxic treatment remains controversial. This retrospective study investigated whether sperm bankers with different medical conditions have worse baseline semen parameters as compared to individuals with non-medical indications for banking.

Methods: With IRB approval, de-identified records were obtained for all episodes of sperm banking performed at the New England Cryogenic Center from January 2004 to May 2017 for one of the following reasons: “future use” (e.g., military deployment, at-risk travel, gender reassignment); infertility; benign disease; and malignancy, further categorized as testicular, other genitourinary (GU), solid non-GU, hematologic, or unspecified. Bankers with prior exposure to chemotherapy or pelvic radiation were excluded. For any banker with multiple specimens, the average of semen parameters across encounters was used. Dependent variables of interest were ejaculatory volume, sperm concentration, percent motility, and total motile sperm count (TMSC), as well as post-thaw motile count. Independent T-test and stepwise multiple linear regressions controlling for age were performed, with the “future use” group as reference for other groups. Additional linear regressions were performed with only significant variables and adjusted for interaction with age where relevant.

Results: 1561 patients met the inclusion criteria. In univariate analyses, infertility was associated with age (P=0.044), motility (P=0.004), and TMSC (P=0.005). Hematologic malignancy was associated with motility (P=0.02) and TMSC (P=0.019). In stepwise multiple linear regressions, benign disease was not associated with any variables, while for the infertility group, all measured semen parameters were decreased (all P<0.004). All subtypes of malignancy were associated with decreased TMSC (all P<0.002). Testicular malignancy was also associated with decreased sperm concentration (P<0.001) and post-thaw motile count (P<0.001); other GU malignancy, with volume (P<0.001); and hematologic malignancy, with motility (P=0.026).

Conclusion: In addition to bankers with known infertility issues, sperm bankers with malignancy, but not benign disease, had worse baseline semen parameters as compared to individuals banking for non-medical reasons. These results can inform patient counseling and consent prior to sperm banking and disease treatment.
POSTER SESSION I

SUNDAY, APRIL 22, 2018
*Poster Session I
11:30 a.m. - 12:30 p.m.
*Not CME Accredited

Oral/Poster# 1
CRISPR-CAS9 MEDIATED KNOCKDOWN OF ODF2 IN PORCINE TESTICULAR SOMATIC CELLS
Taylor Goldsmith BSc, Dennis Webster¹, Dan Carlson PhD¹ and Ina Dobrinski DVM, PhD²
¹Recombinetics Inc; ²University of Calgary
(Presented By: Taylor Goldsmith BSc)

Oral/Poster# 2
INVESTIGATING ZIKA VIRUS PATHOGENESIS ON MALE REPRODUCTION USING A HUMAN 3-DIMENSIONAL TESTICULAR ORGANOID MODEL
Nima Pourhabibi Zarandi MD¹, Daniel Strange², Goral Trivedi³, Anthony Atala MD¹, Colin Bishop PhD¹, Saguna Verma PhD² and Hooman Sadri-Ardekani MD, PhD¹
¹Wake Forest Institute for Regenerative Medicine (WFIRM) and Department of Urology, Wake Forest School of Medicine, Winston Salem, NC; ²Department of Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI
(Presented By: Nima Pourhabibi Zarandi MD)

Oral/Poster# 3
IN VITRO CULTURE OF HUMAN KLINEFELTER SPERMATOGONIAL STEM CELLS
Guillermo Galdon MD¹, Nima Pourhabibi-Zarandi MD¹, Mark Pettenati PhD², Stuart Howards MD¹,4, Stanley Kogan MD FACS¹,5, Anthony Atala MD¹,5 and Hooman Sadri-Ardekani MD PhD¹,5 Wake Forest Institute for Regenerative Medicine; ¹Section of Medical Genetics, Department of Pathology Wake Forest School of Medicine; ²Department of Urology; 4Wake Forest School of Medicine; 5Wake Forest School of Medicine, Wake Forest Institute for Regenerative Medicine
(Presented By: Guillermo Galdon MD)

Oral/Poster# 4
PREGNANCY ESTABLISHED AFTER AUTOLOGOUS GRAFTING OF CRYOPRESERVED TESTICULAR TISSUE FROM PREPUBERTAL RHESUS MACAQUES
Adetunji Fayomi DVM, MVSc¹, Peters Karen BS¹, Meena Sukhwani PhD², Cathy Ramsey BS¹, Carol Hanna PhD¹, Jon Hennebold PhD¹ and Kyle Orwig PhD²
¹Magee-Womens Research Institute, University of Pittsburgh School of Medicine; ²Magee-Womens Reserach Institute University of Pittsburgh School of Medicine, Pittsburgh, PA; ³Assisted Reproductive Technology Core, Oregon National Primate Research Center, Beaverton, OR
(Presented By: Adetunji Fayomi DVM, MVSc)

Oral/Poster# 5
PUBERTAL DEVELOPMENT AFTER TESTICULAR TISSUE BIOPSY FOR FERTILITY PRESERVATION
Aude Braye PhD-student¹, Inge Gies Prof Dr², Veerle Vloeberghs Dr², Alina Ferster Prof Dr², Jutte Van der Werff Ten Bosch Prof Dr³, Herman Tournaye Prof Dr² and Ellen Goossens Prof Dr¹
¹Vrije Universiteit Brussel; ²Universitaair Ziekenhuis Brussel; ³Hôpital Universitaire Des Enfants Reine Fabiola
(Presented By: Aude Braye PhD-student)

Oral/Poster# 6
EPIDIDYMAL SPERM MATURATION – ROLES OF CALCINEURIN, PP1G2, AND GSK3
Souvik Dey PhD¹, Alaa Eisa MS², Suranjana Goswami MS¹, Rahul Bhattacharjee MS¹, Douglas Kline PhD and Srinivasan Vijayaraghavan PhD³
¹Department of Biological Sciences, Kent State University, Kent, Ohio; ²School of Biomedical Sciences, Kent State University, Kent, Ohio
(Presented By: Souvik Dey PhD)

Oral/Poster# 7
ABSENCE OF 14-3-3 EPSILON ALTERS MALE FERTILITY, SPERM COUNT, AND MOTILITY IN MICE
Alaa Eisa¹, Alexander Ignatious¹, Souvik Dey², Sumit Bhutada³, Srinivasan Vijayaraghavan and Douglas Kline²
¹School of Biomedical Science, Kent State University; ²Department of Biological Sciences, Kent State University
(Presented By: Alaa Eisa)

Oral/Poster# 8
A MOUSE GOLGIN PROTEIN, GAMP-210 IS REQUIRED FOR ACROSOME FORMATION BUT NOT FLAGELLA FORMATION
Suheng Ma, Shiyang Zhang MD¹, Wei Li MD², Ting Zhou MD, PhD², Rex Hess PhD⁴, Gregory Pazour PhD⁵ and Zhibing Zhang MD, PhD¹
¹Virginia Commonwealth University; ²Virginia Commonwealth Universtiy; ³Wuhan University of Science and Technology; ⁴University of Illinois; ⁵University of Massachusetts Medical School
(Presented By: Suheng Ma)
POSTER SESSION I

Oral/Poster# 9
NEW INSIGHTS INTO THE VITAMIN D CONCENTRATION AND ITS ASSOCIATION WITH SEMEN QUALITY IN MALE SUBJECTS
Inari Ciccone MSc Student¹, Juliana Pariz MD, PH¹, Elaine Costa MD, PH², Parviz Gharagozloo MD, PH³, John Aitken PHD4 and Jorge Hallak PHD5
¹Department of Urology. University of Sao Paulo. Brazil/ Androscience — High Complexity Clinical and Research Andrology Laboratory. Brazil; ²Department of Endocrinology. University of Sao Paulo. Brazil/ Androscience — High Complexity Clinical and Research Andrology Laboratory. Brazil; ³CellOxess LLC; ⁴CellOxess LLC/University of New Castle, Callaghan, NSW Australia; ⁵Department of Urology. University of Sao Paulo. Brazil/ Androscience — High Complexity Clinical and Research Andrology Laboratory. Brazil/ CellOxess LLC
(Presented By: Inari Ciccone MSc Student)

Oral/Poster# 10
MALIGNANCY NEGATIVELY ASSOCIATED WITH BASELINE SEMEN PARAMETERS AMONG SPERM BANKERS
Rena Xu MD, MBA¹, Grace Centola PhD² and Cigdem Tanrikut MD¹
¹Massachusetts General Hospital; ²New England Cryogenic Center
(Presented By: Rena Xu MD, MBA)

Poster# 11
GEIMINI STUDY: FINE-MAPPING THE GENETIC ARCHITECTURE OF SEVERE MALE INFERTILITY
Liina Nagirnaja¹, Jannette Rusch¹, Alexandro Lopes³, Kristian Almstrup⁴, Brendan Houston⁵, Ewa Rajpert-De Meyts⁴, Maris Laan⁶, Moira O’Bryan⁵, Kenneth I. Aston⁷, Donald F. Conrad⁸ and the GEMINI Consortium¹
¹Washington University School of Medicine, St. Louis, United States; ²Instituto de Investigação e Inovação em Saúde, Universidade do Porto; ³Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; ⁴Department of Growth and Reproduction, Rigshospitalet and University of Copenhagen, Denmark; ⁵The School of Biological Sciences, Monash University, Australia; ⁶Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia; ⁷University of Utah School of Medicine, Salt Lake City, United States
(Presented By: Liina Nagirnaja)

Poster# 12
COP9 SIGNALOSOME COMPLEX SUBUNIT 5 (COPS5), AN IFT20 BINDING PARTNER, IS ESSENTIAL TO MAINTAIN MALE GERM CELL SURVIVAL AND ACROSOME BIOGENESIS
Qian Huang, Jing Zeng MD, PhD¹, Hong Liu MD², Wei Li MD³, Shiyang Zhang MD⁴, Ling Zhang MD, PhD⁵, Ting Zhou MD, PhD⁵, Rex Hess PhD⁴, Ruggero Pardi PhD⁵ and Zhbing Zhang MD, PhD¹
¹Virginia Commonwealth University; ²Virginia Commonwealth Universtity; ³Wuhan University of Science and Technology; ⁴University of Illinois; ⁵San Raffaele University School of Medicine
(Presented By: Qian Huang)

Poster# 13
ABERRANT Y-BOX RNA-BINDING PROTEIN EXPRESSION IS A CANDIDATE FOR MATURATION ARREST AZOOSPERMIA
Ryan Flannigan MD, Anna Mielnik MSc, Alex Bolyakov MSc, Russell Hayden MD MSc, Peter Schlegel MD and Darius Paduch MD PhD
Weill Cornell Medicine
(Presented By: Ryan Flannigan MD)

Poster# 14
AN EPIMUTATION IN MSH5 CONTRIBUTES TO MALE INFERTILITY
Boryana Zhelyazkova BA¹ and Dolores J. Lamb PhD²
¹Baylor College of Medicine; ²Center for Reproductive Medicine, Department of Molecular and Cellular Biology, Scott Department of Urology, Baylor College of Medicine, Houston, Texas
(Presented By: Boryana Zhelyazkova BA)

Poster# 15
THE ROLE OF ADCY2 COPY NUMBER VARIANTS IN CONGENITAL GENITOURINARY ANOMALIES
Marisol O’Neill MS, Nannan Thirumavalavan MD, Kunj Sheth MD, Jeffrey White MD PhD and Dolores Lamb PhD
Baylor College of Medicine
(Presented By: Marisol O’Neill MS)

Poster# 16
SPERM PRODUCTION IN DE NOVO FORMED SEMINIFEROUS TUBULES FROM ALLOGENEIC TRANSPLANTED TESTICULAR CELLS IN RHESUS MONKEY TESTIS
Gunapala Shetty PhD¹, Jennifer Mitchell VMD¹, Truong Lam BS¹, Zhuang Wu MS¹, Jie Zhang PhD¹, Lori Hill DVM¹, Ramesh Tailor PhD¹, Karen Peters MS², Cecilia Penedo PhD², Kyle Orwig PhD² and Marvin Meistrich PhD¹
¹University of Texas M.D. Anderson Cancer center; ²Magee-Womans Research Institute, University of Pittsburgh School of Medicine; ³University of California, Davis
(Presented By: Gunapala Shetty PhD)
POSTER SESSION I

Poster# 17
IN VIVO AND IN VITRO EFFECTS OF THE ENDOCRINE DISRUPTORS GENISTEIN AND MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) ON RODENT MACROPHAGE INFLAMMATORY RESPONSES
Vanessa Brouard PhD¹, Shahrzad Ghazisaeidi Master², Berenice Collet Master², Annie Boisvert Master² and Martine Culty PhD¹
¹University of Southern California; ²McGill University, Montreal, Canada
(Presented By: Vanessa Brouard PhD)

Poster# 18
A NUCLEOCYTOPLASMIC TRANSPORT PROTEIN IMPLICATED IN BMP SIGNALING CONTROL IS REQUIRED FOR FERTILITY
Kate Loveland PhD¹, Julia Young PhD², Diana Micati BSci (Hons)³, Elizabeth Richards BSSci³ and Eileen McLaughlin PhD⁴
¹Hudson Institute of Medical Research and Monash University; ²Monash University; ³Hudson Institute of Medical Research; ⁴University of Auckland and University of Newcastle
(Presented By: Kate Loveland PhD)

Poster# 19
PIPERINE PROMOTES PUBERTAL LEYDIG CELL DEVELOPMENT BUT INHIBITS SPERMATOGENESIS IN RATS
Xianwu Chen MD, Fei Ge MD, Qingquan Lian PhD and Ren-shan Ge PhD
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
(Presented By: Xianwu Chen MD)

Poster# 20
HUMAN SPERMATOGONIAL STEM CELL CULTURE USING TISSUE-DERIVED EXTRACELLULAR MATRICES
Sherin David MS¹, Mark Murdock BS², Sarah Munyoki BS³, Kathrin Gassei PhD⁴, Stephen Badylak DVM, MD, PhD⁵ and Kyle Orwig PhD⁶
¹Department of Obstetrics, Gynecology and Reproductive Sciences, Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, Magee-Womens Research Institute, Pittsburgh, PA; ²Cellular and Molecular Pathology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, McGowan Institute for Regenerative Medicine, Pittsburgh, PA; ³Department of Obstetrics, Gynecology and Reproductive Sciences, Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, Integrative Systems Biology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, Magee-Womens Research Institute, Pittsburgh, PA; ⁴Magee-Womens Research Institute, Pittsburgh, PA; ⁵Cellular and Molecular Pathology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, McGowan Institute for Regenerative Medicine, Pittsburgh, PA, (Presented By: Sherin David MS)

Poster# 21
DEVELOPMENT OF SPERMATOGENESIS IN THREE-DIMENSION CULTURE FROM SPERMATOGONIAL CELLS OF BUSULFAN-TREATED IMMATURE MICE
Ali AbuMadigem MSc¹, Ronnie Solomon MSc¹, Alina Stepanovsky MSc², Joseph Kapelushnik MD³, Eitan Lunenfeld MD⁴ and Mahmoud Huleihel PhD⁵
¹The Shraga Segal Dep. of Microbiology, Immunology and Genetics, The Center of Advanced Research and Education in Reproduction (CARER), Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; ²The Shraga Segal Dep. of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; ³Dep. of Pediatric Oncology and Dep. of Hematology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; ⁴Fertility and IVF Unit, Dep. OB/GYN, Soroka Medical Center and The Center of Advanced Research and Education in Reproduction (CARER), Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.; ⁵The Shraga Segal Dep. of Microbiology, Immunology and Genetics, The Center of Advanced Research and Education in Reproduction (CARER), Faculty of Health Sciences, The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev
(Presented By: Mahmoud Huleihel PhD)

Poster# 22
A COMBINATION OF GINGER, PAULLINIA CUPANA, MUIRA PUAMA AND L-CITRULLINE ACTIVATES THE INOS-SGC-CGMP PATHWAY IN CAVERNOSAL SMOOTH MUSCLE CELL CULTURE
Monica G. Ferrini Ms, PhD¹, Andrea Abraham BS¹, Sabine Nguyen DO¹, Jorge N. Artaza MS, PhD² and Jacob Rajfer MD³
¹Charles R. Drew University; ²David Geffen UCLA School of Medicine
(Presented By: Monica G. Ferrini Ms, PhD)

Poster# 23
CIGARETTE SMOKE-INDUCED SPERM DNA METHYLATION (DNAME) CHANGES IN MICE AND PARTIAL RECOVERY FOLLOWING SMOKING CESSION
Kenneth Aston PhD¹, Patrick Murphy PhD², Timothy Jenkins PhD², Douglas Carrell PhD² and Bradley Cairns PhD²
¹Department of Surgery, University of Utah School of Medicine; ²Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine
(Presented By: Kenneth Aston PhD)
POSTER SESSION I

Poster# 24
ABSCALON OF THE CONSERVED GATA BINDING MOTIF IN THE PROMOTER SIGNIFICANTLY IMPAIRS EXPRESSION
Marie France Bouchard PhD¹, Francis Bergeron MS² and Robert Viger PhD³,⁴
¹Reproduction, Mother and Child Health, Centre de recherche du CHU de Québec-Université Laval and Centre de recherche en reproduction, développement et santé intergénérationnelle (CRDSI), Québec, Canada; ²Department of Obstetrics, Gynecology, and Reproduction, Faculty of Medicine, Laval University, Québec, Canada
(Presented By: Marie France Bouchard PhD)

Poster# 25
FUNCTIONAL COOPERATION BETWEEN THE TRANSCRIPTION FACTORS C/EBP? AND CJUN ON THE MOUSE INHIBIN BET
A SUBUNIT GENE IN LEYDIG CELLS
Nicholas Robert MSc¹, Gabriel Garon MSc² and Jacques J. Tremblay PhD³
¹CHU de Quebec Research Centre - Laval University; ²CHU de Quebec Research Centre - Laval University
(Presented By: Nicholas Robert MSc)

Poster# 26
LUMINAL ATP AND ADENOSINE MODULATE V-ATPASE-DEPENDENT PROTON SECRETION IN EPIDIDYMAL CLEAR CELLS
Maria Agustina Battistone PhD, Maria Merkulova PhD, Maria Peralta Bachelor, Nicolas DaSilva PhD, Dennis Brown PhD and Sylvie Breton PhD
Massachusetts General Hospital/Harvard Medical School
(Presented By: Maria Agustina Battistone PhD)

Poster# 27
SPERM CAPACITATION IS ASSOCIATED WITH PHOSPHORYLATION OF THE TESTIS-SPECIFIC RADIAL SPOKE PROTEIN RSPH6
Bidur Paudel¹ and Pablo E. Visconti²
¹University of Massachusetts, Amhe st; ²Umass, Amhe st
(Presented By: Bidur Paudel)

Poster# 28 - WITHDRAWN

Poster# 29
ROLE OF THE ARYL HYDROCARBON RECEPTOR IN THE DISTORTION OF THE SEX RATIO IN EMBRYOS SIRE BY TCDD-
EXPOSED MALE MICE
Kristin Bircsak PhD¹, Andrew Prantner PhD², Latresa Copes MS³ and George Gerton PhD⁴
¹Center of Excellence in Toxicology and Center for Research on Reproduction and Women’s Health, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; ²Center for Research on Reproduction and Women’s Health, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
(Presented By: George Gerton PhD)

Poster# 30
PATTERNS OF EPIGENETIC INSTABILITY PROVIDE NOVEL INSIGHT INTO HUMAN SPERM FUNCTION AS WELL AS
POTENTIAL DIAGNOSTIC UTILITY
Tim Jenkins PhD, Kenneth Aston PhD and Douglas Carrell PhD
University of Utah
(Presented By: Tim Jenkins PhD)

Poster# 31
DIFFERENTIAL RNA EXPRESSION IN THE SPERM OF COUPLES WITH RECURRENT PREGNANCY LOSS
Luke Simon PhD¹, Tim Jenkins PhD², Ki Aston PhD² and Douglas Carrell PhD³
¹University of Utah; ²Department of Surgery (Urology), University of Utah School of Medicine, Salt Lake City, UT, USA; ³Department of Surgery (Urology), Department of Human Genetics, Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT, USA
(Presented By: Luke Simon PhD)

Poster# 32
THE COMBINED EFFECT OF BMI AND AGING ON DNA METHYLATION SIGNATURES IN HUMAN SPERM
Dallin Broberg BS, Tim Jenkins PhD, Kenneth Aston PhD and Douglas Carrell PhD
University of Utah
(Presented By: Dallin Broberg BS)

Poster# 33
ENVIRONMENTAL PHENOL AND PARABEN EXPOSURE IS NEAR UBIQUITOUS AND AFFECTS SERUM TESTOSTERONE
LEVELS IN HEALTHY ADULT MEN
Joseph Gabrielsen MD, PhD and Dolores Lamb PhD
Center for Reproductive Medicine, Baylor College of Medicine
(Presented By: Joseph Gabrielsen MD, PhD)
POSTER SESSION I

Poster# 34
TIMING OF SPERM CAPACITATION VARIES AMONG MEN AND IS CONSISTENT WITHIN MEN
G. Charles Ostermeier PhD, Cristina Cardona PhD¹, Melissa A. Moody MS¹, Alana J. Simpson BS¹, Romeo Mendoza BS¹ and Alexander J. Travis VMD, PhD²
¹Androvia LifeSciences LLC; ²Cornell University College of Veterinary Medicine
(Presented By: G. Charles Ostermeier PhD)

Poster# 35
ABNORMAL EXPRESSION, LOCALIZATION, AND TRAFFICKING OF MICRO-RNAS IN MEN WITH NONOBSTRUCTIVE AZOOSPERMIA
Russell Hayden MD, Anna Mielnik MS, Ryan Flannigan MD, Alexander Bolyakov MS and Darius Paduch MD, PhD
WCM
(Presented By: Russell Hayden MD)

Poster# 36
ANALYZING THE FUNCTION OF THE CAENORHABDITIS ELEGANS GENE M05D6.2, AN ORTHOLOG OF HUMAN T-COMPLEX PROTEIN 11 (TCP11), IN SPERM FUNCTION AND FERTILITY
Amber Jacob, Emily Lopes BS, Danielle Cooley and Matthew Marcello PhD
Pace University
(Presented By: Amber Jacob)

Poster# 37
GENOME-WIDE STUDY OF NONOBSTRUCTIVE AZOOSPERMIA: NOVEL GENES AND NEW DIAGNOSTIC OUTLOOK
Alexander Yatsenko PhD¹, Nijole Pollock BS², Huaiyang Jiang MD², Marta Olszewska PhD³, Tomas Jaffe MD4, Svetlana Yatsenko MD5, Joseph Sanfilippo MD5, Aleks Rajkovic MD, PhD5 and Maciej Kurpisz MD, PhD³
¹MWRI; ²MWRI, University of Pittsburgh, PA; ³Institute of Human Genetics, Poznan, Poland; 4Department of Urology, University of Pittsburgh, PA; 5Department of OB/GYN and Reproductive Science, University of Pittsburgh, PA
(Presented By: Alexander Yatsenko PhD)

Poster# 38
ANALYSIS OF SPERMATOGONIAL QUANTITY IN PEDIATRIC TESTES: ESTABLISHING REFERENCE VALUES AND A CLINICAL TOOL TO EVALUATE IMMATURE TESTES
Stanley Kogan MD¹, Abinav Udaiyar², Heather Barber³, Demetri Hodges³, Guillermo Galdon MD², Nima Pourhabibi Zarandi MD², Kimberly Stogner-Underwood MD³, Shadi Quassem MD4, Kimberly Stogner-Underwood MD³, Shadi Quassem MD4, Anthony Atala MD² and Hooman Sadri-Ardekani MD, PhD²
¹Wake Forest University School of Medicine; ²WFIRM; ³Dept of Pathology, WFUBMC; 4Dept of Pathology, WFUBMC
(Presented By: Stanley Kogan MD)

Poster# 39
DIRECT QUANTIFICATION OF FGFR2 MUTATION LEVELS IN SPERM OF CANCER PATIENTS AFTER CHEMOTHERAPY OR RADIOThERAPY
Marvin Meistrich PhD¹, Geoffrey Maher², Marie Bernkopf², Andrew Wilkie² and Anne Gorici²
¹University of Texas M.D. Anderson Cancer Center, Houston, TX, USA; ²MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK
(Presented By: Marvin Meistrich PhD)

Poster# 40
AMHR2-CRE-MEDIATED GLOBAL TSPO KNOCKOUT AND ITS ADVERSE EFFECT ON EMBRYONIC DEVELOPMENT, NEUTRAL LIPID ACCUMULATION AND REDUCED CIRCULATING TESTOSTERONE LEVELS IN THE ADULT
Jinjiang Fan PhD¹, Enrico Campioli PhD² and Vassilios Papadopoulos DPharm, PhD³
¹The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; ²Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, California, USA
(Presented By: Jinjiang Fan PhD)
POSTER SESSION II

MONDAY, APRIL 23, 2018
*Poster Session II
11:30 a.m. - 12:30 p.m.
Location: The Nines Ballroom PreFunction
*Not CME Accredited

Poster# 41
ROLE OF MITOCHONDRIAL TRANSLOCATOR PROTEIN (TSPO) MODIFICATIONS AND PROTEIN-PROTEIN INTERACTIONS IN MEDITATING THE EFFECT OF HORMONES AND THE TSPO DRUG LIGAND XBD173 TSPO ON LEYDIG CELL STEROID PRODUCTION
Yasaman Aghazadeh PhD¹, Enrico Campioli DPharm, PhD¹, Barry Zirkin PhD² and Vassilios Papadopoulos DPharm, PhD³
¹Research Institute of the McGill University Health Centre and Department of Medicine, McGill University, Montreal, Quebec, H4A 3J1, Canada;
²Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205, USA;
³Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, California 90089, USA
(Presented By: Vassilios Papadopoulos DPharm, PhD)

Poster# 42
INTRACELLULAR CALCIUM CHANGES IN MOUSE AND HUMAN SPERM INDUCED BY MEMBRANE DEPOLARIZATION AND PH CHANGES.
Juan Ferreira BS¹, Pascale Lybaert PhD², Aluet Borrego Alvarez MS¹, Julio Chavez PhD¹, Mariana Ford BS¹, Joan Riley PhD¹ and Celia M Santi MD, PhD¹
¹Washington University; ²Université Libre de Bruxelles.; ³Universidad de la Republica (UdelaR)
(Presented By: Juan Ferreira BS)

Poster# 43
TARGETING HISTONE H3 LYSINE DEMETHYLASE KDM1A WITH THE DRUG GSK2879552 INHIBITS MALE FERTILITY THROUGH GERM CELL LOSS IN THE TESTIS
Sarayu Ratnam PhD, Asha Varghese BS and Christopher Payne PhD
Northwestern University Feinberg School of Medicine
(Presented By: Christopher Payne PhD)

Poster# 44
PRIMARY CILIA: CELL ANTENNAS AND SIGNALLING HUBS IN THE EPIDIDYMIS.
Agathe Bernet, Olivia Jerzymski, Maira Bianchi Rodrigues Alves, Christian Roy, Laura Girardet, Alexandre Bastien, Claude Robert, Denis Soulet and Clemence Belleannee PhD
U. Laval
(Presented By: Clemence Belleannee PhD)

Poster# 45
TITLE: DELETION OF KANK1 IN MALE MICE IS ASSOCIATED WITH DECREASED SPERMATOGENESIS
Nannan Thirumavalavan MD¹, Marisol A. O’Neill MS², Meade Haller PhD³, Cenk Cengiz BS¹, Joshua Moore MS4, Jeffrey White MD, PhD5, Kunj R. Sheth MD5 and Dolores J. Lamb PhD²
¹Center for Reproductive Medicine, Scott Department of Urology, Baylor College of Medicine; ²Center for Reproductive Medicine, Scott Department of Urology and Department of Molecular and Cellular Biology, Baylor College of Medicine; ³Center for Reproductive Medicine, Scott Department of Urology and Department of Molecular and Cellular Biology, Baylor College of Medicine; 4Scott Department of Urology, Department of Molecular and Cellular Biology, Baylor College of Medicine; 5Center for Reproductive Medicine, Scott Department of Urology, Texas Children’s Hospital, Baylor College of Medicine
(Presented By: Nannan Thirumavalavan MD)

Poster# 46
HIGH-QUALITY SPERM RNA MOLECULAR BIOMARKERS TO ASSESS FERTILITY AND ENVIRONMENTAL TOXICANT EXPOSURES
Enrica Bianchi PhD, Angela Stermer PhD, Kim Boekelheide MD, PhD, Mark Sigman MD, Susan Hall and Kathleen Hwang MD
Brown University
(Presented By: Enrica Bianchi PhD)

Poster# 47
VALIDATION OF CELLVISION DISPOSABLE 100 MICROMETER HEMOCYTOMETER WITH IMPROVED NEUBAUER RULING
Lars Björndahl MD PhD, Kristina Magnusson BMS and Rebecka Holmberg BMS PhD
ANOVA, Karolinska University Hospital and Karolinska Institutet
(Presented By: Lars Björndahl MD PhD)
POSTER SESSION II

Poster# 48
EPITHELIA SPECIFIC TGFβ1 TARGETING INDUCES LEUKOCYTOSIS IN MOUSE EPIDIDYMIS
Fernando Pierucci-Alves DVM¹, Bruce Schultz PhD¹ and Sherry Fleming PhD²
¹Dept of Anatomy & Physiology, Kansas State University; ²Division of Biology, Kansas State University
(Presented By: Fernando Pierucci-Alves DVM)

Poster# 49
IN UTERO EXPOSURE TO BENZYLBUTYL PHTHALATE DISRUPTS FETAL LEYDIG CELL DEVELOPMENT AND INDUCES MULTINUCLEATED GONOCYTES IN RATS
Zina Wen MS¹, Fei Ge MD², Renshan Ge MD² and Ying Zhong MD¹
¹Jinjiang Maternity and Child Health Hospital; ²The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Zina Wen MS)

Poster# 50
AXONEMAL DYNEIN LIGHT INTERMEDIATE POLYPEPTIDE 1 FORMS A COMPLEX WITH PACRG IN THE MANCHETTE FOR CARGO TRANSPORT
Wei Li, David Williams PhD¹ and Zhibing Zhang MD, PhD²
¹University of North Carolina; ²Virginia Commonwealth University
(Presented By: Wei Li)

Poster# 51
CHARACTERIZATION OF MOUSE INTRAFLAGELLAR TRANSPORTER PROTEIN IFT74 IN MALE GERM CELLS
Lin Shi, Wei Li MD¹, Shiyang Zhang MD², Ting Zhou MD, PhD³, Gregory Pazour PhD³ and Zhibing Zhang MD, PhD²
¹Virginia Commonwealth University; ²Virginia Commonwealth University; ³University of California, San Diego; ⁴University of Massachusetts Medical School
(Presented By: Lin Shi)

Poster# 52
EFFECTS OF TRIPITOLIDE ON THE EXPRESSION OF ANDROGEN RECEPTOR IN HUMAN PROSTATE CELLS AND ITS MECHANISMS IN TRANSCRIPTION LEVEL
Xiao Gu MD andn PhD¹, Bide Liu MS¹, Wei Li PhD², Jin Yang MS¹ and Hong Zhao MHand PhD³
¹The Clinical Medical College at Yangzhou University; ²Yangzhou University; ³University of Louisville
(Presented By: Xiao Gu MD and PhD)

Poster# 53
SYSTEMATIC IN-DEPTH PROTEOMIC ANALYSES REVEAL NOVEL PROTEINS ARE ENRICHED WITHIN HUMAN AND MOUSE TESTIS MITOCHONDRIA-ASSOCIATED MEMBRANES(MAM)
Shuiqiao Yuan PhD
Huazhong University of Science and Technology
(Presented By: Shuiqiao Yuan PhD)

Poster# 54
KCTD13 GENE DOSAGE CHANGES RESULT IN PENILE AND TESTICULAR ANOMALIES VIA AN ABERRANT ANDROGEN RECEPTOR SIGNALING
Abhishek Seth MD, Armando Rivera PhD, In-seon Choi PhD, Shaye Lewis PhD, Carolina Jorgez PhD and Dolores Lamb PhD
Baylor College of Medicine
(Presented By: Presented by Carolina Jorgez, PhD)

Poster# 55
FLOW CYTOMETRY ANALYSIS REVEALED A RAPID INCREASE IN INTRACELLULAR CALCIUM IN A SUBPOPULATION OF MOUSE SPERM DURING CAPACITATION
Guillermina Luque PhD, Tomas Dalotto Moreno PhD¹, David Martin Hidalgo PhD², Carla Ritagliati PhD³, Lis Puga Molina Msc¹, Ana Romarowski PhD², Paula A Balestrini Msc², Liza J Schiavi Ehrenhausen Student¹, Nicolas Gilio Student¹, Dario Krupf PhD³, Pablo E Visconti PhD³ and Mariano G Buffone PhD²
¹IBYME; ²VASCU UMASS; ³IBR
(Presented By: Guillermina Luque, PhD)

Poster# 56
ROLE OF SERINE PROTEASES (PRSS50) IN SPERMATOGENESIS
Minerva Solis, Joshua Moore MS, Juan Bounrat PhD, Adam Szafran MD PhD, Jason Scovell BS and Carolina Jorgez PhD
Baylor College of Medicine
(Presented By: Minerva Solis)
POSTER SESSION II

Poster# 57
SUBCLINICAL GENITOURINARY INFECTION SUSPECTED BY PHYSICAL EXAMINATION INCREASES REACTIVE OXYGEN SPECIES IN SEMINAL PLASMA: AN INITIAL REPORT
Jorge Hallak MD, PhD¹,²,³, Rosa Monteiro BSc¹,²,³ and Juliana Pariz PhD¹,²,³,⁴
¹Androscience – Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Laboratory, Sao Paulo, Brazil; ²Dept. of Urology, Universidade de Sao Paulo (USP), Brazil; ³Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil; ⁴Methodist University of Sao Paulo, Brazil
(Presented By: Jorge Hallak MD, PhD)

Poster# 58
SNP AND ADAR MODIFICATION ANALYSIS OF MICRO-RNA'S IN 54 MEN WITH MALE-FACTOR INFERTILITY
Russell Hayden MD, Ryan Flannigan MD, Alexander Bolyakov MS, Anna Mielnik MS and Darius Paduch MD, PhD
WCM
(Presented By: Russell Hayden MD)

Poster# 59
A NEW SPERM EF-HAND PROTEIN, EFCAB9, IS ASSOCIATED WITH CATSPER CHANNEL AND ESSENTIAL FOR MALE FERTILITY
Jae Yeon Hwang PhD and Jean-Ju Chung PhD
Yale School of Medicine
(Presented By: Jean-Ju Chung PhD)

Poster# 60
PHTHALATES AFFECT HUMAN TESTIS RETINALDEHYDE DEHYDROGENASE 1 MEMBER A1 AS THE POTENTIAL ENDOCRINE DISRUPTORS
Erpo Tian MD¹, Fei Ge MD², Renshan Ge MD² and Ying Zhong MD¹
¹Jinjiang Maternity and Child Health Hospital; ²The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Erpo Tian MD)

Poster# 61
ADJUDIN-LOADED NANOCAPSULES IN CONTRACEPTION OF MALE RATS
Chao Li Phd, Baiping Mao PhD and Renshan Ge Professor
(Presented By: Chao Li Phd)

Poster# 62
SPERM DNA DAMAGE: CONSEQUENCES OF THE IMPACT OF YOGIC COGNITIVE BEHAVIOR PRACTICES
Shilpa Bisht MSc, Priyanka Chaurasiya MSc, Rohit Kumar Khetan MSc, Madhuri Tolahunase MSc and Rima Dada MD, PhD
Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Shilpa Bisht MSc)

Poster# 63
GRANULOCYTE COLONY-STIMULATING FACTOR REGULATES DIFFERENTIATION OF STEM LEYDIG CELLS
Linchao Li MD, Yiyan Wang MD, Qingquan Lian PhD and Ren-Shan Ge PhD
The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University
(Presented By: Linchao Li MD)

Poster# 64
A BRIEF EXPOSURE TO PERFLUOROOCTANE SULFONATE IMPAIRS STEM LEYDIG CELLS IN THE ADULT RAT TESTIS
Baiping Mao Ph D, Qingquan Lian PhD and Ren-Shan Ge MD
The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, 109 Xueyuan West Road, Wenzhou, Zhejiang 325027, PR, China
(Presented By: Baiping Mao Ph D)

Poster# 65 - WITHDRAWN

Poster# 66
HYPERCHOLESTEROLEMIA INDUCED SPERM DAMAGE IN RAT AND THERAPEUTIC POTENTIAL OF MUCUNA PRURIENS (LINN.)
Seppan Prakash PhD, Murgesh Anuradha PhD, Mohammed Ibrahim PhD, Mohanraj Karthik Ganesh PhD, Lakshmanan Ganesh PhD, Premavathy Dinesh MSc, Muthu Sakthi Jothi MSc and Wungpam Shimray Khayimni MSc
University of Madras
(Presented By: Seppan Prakash PhD)
POSTER SESSION II

Poster# 67
TESTICULAR TISSUES FROM PATIENTS WITH NON-OBSTRUCTIVE AZOOSPERMIA EXHIBIT A DIFFERENTIAL PIRNAS EXPRESSION PROFILE BETWEEN SUCCESSFUL AND UNSUCCESSFUL SPERMATOZOA RETRIEVAL
Xunbin Huang MD, Congcong Cao BS and Na Fang Bs
Family Planning Research Institute, Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology
(Presented By: Xunbin Huang MD)

Poster# 68 - WITHDRAWN

Poster# 69
GUO’S SINGLE-ARMED SUTURE TECHNIQUE FOR MICROSCOPICAL VASEOEPIDIDYMOSTOMY
Yiming Yuan MD & PhD, Hongen Lei MD, Zhichao Zhang MD, Jing Peng MD, Wanshou Cui MD and Zhongcheng Xin MD
Andrology Center & Urology Department, Peking University First Hospital
(Presented By: Yiming Yuan MD & PhD)

Poster# 70
NANOTECHNOLOGY STRUCTURED WATER INCREASES SERUM TESTOSTERONE LEVEL IN HYPOGONADAL MEN
Ali Kamal M. Sami MD, Diar Nameed Bajalan MD and Mohammed Abed Kadum Hassan MD
(Presented By: Ali Kamal M. Sami MD)

Poster# 71
EFFECT OF LIFESTYLE FACTORS ON SPERMATOZOAL HEALTH IN RECURRENT PREGNANCY LOSS
Rima Dada MD, PhD¹, Vidhu Dhawan MD¹, Manoj Kumar PhD¹, Dipika Deka MD², Neena Malhotra MD², Neeta Singh MD² and Vatsal Dadhwal MD²
¹Laboratory for Molecular Reproduction & Genetics, Dept. of Anatomy, AIIMS; ²Dept. of Obstetrics & Gynaecology, AIIMS
(Presented By: Rima Dada MD, PhD)

Poster# 72
PEYRONIE’S DISEASE IS ASSOCIATED WITH INCREASED IMMUNE REACTIVITY: ANALYSIS OF UNITED STATES CLAIMS DATA
Taylor Kohn MPhil¹, Daniel Pichardo², William Meeks², Larry Lipshultz MD¹ and Alexander Pastuszak MD PhD¹
¹Baylor College of Medicine; ²Department of Data Management & Statistical Analysis, American Urological Association; ³Center for Reproductive Medicine & Scott Department of Urology, Baylor College of Medicine
(Presented By: Alexander Pastuszak MD PhD)

Poster# 73
INCREASED RISK OF PEYRONIE’S DISEASE IN MEN TAKING ANTIPSYCHOTIC, ANTIDEPRESSANT, AND ANTIVIRAL MEDICATIONS
Alexander Pastuszak MD PhD¹, Taylor Kohn MPhil², Daniel Pichardo³, William Meeks³ and Larry Lipshultz MD¹
¹Center for Reproductive Medicine & Scott Department of Urology, Baylor College of Medicine; ²Baylor College of Medicine; ³Department of Data Management & Statistical Analysis, American Urological Association
(Presented By: Alexander Pastuszak MD PhD)

Poster# 74
EPIDERMAL GROWTH FACTOR STIMULATES PROLIFERATION OF RAT STEM AND PROGENITOR LEYDIG CELLS VIA UPREGULATING CCND1
Fei Ge MD¹, Yiyian Wang MD², Qingquan Lian PhD² and Ren-shan Ge PhD²
¹The Second Affiliated Hospital of Wenzhou Medical University; ²The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Fei Ge MD)

Poster# 75
PILOT STUDY OF 3D PHOTOGRAPHY FOR ASSESSMENT OF PENILE DEFORMITY IN MEN WITH PEYRONIE’S DISEASE (PD)
Denise Asafu-Adjei MD, MPH, Ezra Margolin MD¹, Doron Stember MD² and Peter J. Stahl MD³
¹Columbia University Medical Center/New York Presbyterian; ²The Mount Sinai Hospital
(Presented By: Denise Asafu-Adjei MD, MPH)

Poster# 76
YOGA OPTIMIZED NEUROPLASTICITY AND REGULATORY FEEDBACKS IMPROVE SEMENOGRAM AND SPERM QUALITY IN INFERTILE MEN WITH MAJOR DEPRESSIVE DISORDER: A RANDOMIZED CONTROLLED TRIAL.
Madhuri Tolahunase MSc¹, Rajesh Sagar MD², Priyanka Chaurasia MSc¹ and Rima Dada MD, PhD¹
¹Lab for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.; ²Department of Psychiatry, All India Institute of Medical Sciences, New Delhi, India.
(Presented By: Madhuri Tolahunase MSc)
POSTER SESSION II

Poster# 77
IMPROVING REPRODUCTIVE EFFICIENCY IN THE BULL: WILL REDUCING ENDOGENOUS ESTRADIOL INCREASE SERTOLI CELL PROLIFERATION?
Kimberly Miller and Trish Berger PhD
Department of Animal Science
(Presented By: Kimberly Miller)

Poster# 78
NOVEL ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY PROFILING OF ANDROGENOME IN HUMAN SALIVA
Nilesh Gaikwad PhD¹ and Prasanth Surampudi MD²
¹Gaikwad Steroidomics Laboratory; ²University of California, Davis
(Presented By: Nilesh Gaikwad PhD)

Poster# 79
PORCINE PERIOVULATORY OVIDUCTAL FLUID INHIBITS THE CAMP/PKA PATHWAY DURING SPERM CAPACITATION IN MICE
Maria Gracia Gervasi PhD¹, Pablo Visconti PhD¹ and Carmen Matás DVM-PhD²,³
¹Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst.; ²Department of Physiology, Faculty of Veterinary Science, International Excellence Campus for Higher Education and Research “Campus Mare Nostrum”, University of Murcia, Murcia, Spain; ³and Institute for Biomedical Research of Murcia (IMIB-Arrixaca), Murcia, Spain.
(Presented By: Maria Gracia Gervasi PhD)

Poster# 80
CHANGES IN O-GLCNACYLATION DURING EPIDIDYMAL SPERM MATURATION
Maria Gracia Gervasi PhD, Darya Tourzani BS and Pablo Visconti PhD
Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst.
(Presented By: Maria Gracia Gervasi PhD)
POSTER SESSION III

TUESDAY, APRIL 24, 2018
*Poster Session III
11:15 a.m. - 12:15 p.m.
Location: The Nines Ballroom PreFunction
*Not CME Accredited

Poster# 81
THE PSYCHOLOGIC AND EMOTIONAL IMPACT OF SUB-FERTILITY ON MEN
Luriel Smith-Harrison MD, Abbey Kruper PsyD and Jay Sandlow MD
Medical College of Wisconsin
(Presented By: Luriel Smith-Harrison MD)

Poster# 82
THE OPTIMAL SURGICAL TIMING FOR FOURNIER’S GANGRENE
Ta-Yao Tai MD, Tsung-Yen Lin MD and Yung-Ming Lin PhD
National Cheng Kung University Hospital
(Presented By: Ta-Yao Tai MD)

Poster# 83
METABOLIC PROFILE OF GENDER DYSPHORIC PERSONS IN A LONG-TERM TREATMENT WITH CROSSEX HORMONES
Stella Santiago MD¹, Henrique Cecotti MD¹, Flavia Cunha Doctor¹, Sorahia Domenice Doctor¹, Berenice Mendonca Professor¹ and Elaine Costa MD, PhD, Prof²
¹Division of Clinical Endocrinology, Hospital das Clinicas, Department of Endocrinology of University of Sao Paulo School of Medicine, Sao Paulo, Brazil; ²Division of Clinical Endocrinology, Hospital das Clinicas, Department of Endocrinology of University of Sao Paulo School of Medicine, Sao Paulo, Brazil/ Androciense
(Presented By: Elaine Costa MD, PhD, Prof)

Poster# 84
MICROSATELLITE INSTABILITY IN SELECTED GENES IMPLICATED IN MALE INFERTILITY
Jyoti Sharma MTech¹, Prakash Chand Sharma MSc, PhD², Rohit Kumar Khetan MSc¹, Priyanka Chaursasia MSc¹, Rajeev Kumar MD³ and Rima Dada MD, PhD¹
¹Laboratory of Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, Delhi, India; ²University School of Biotechnology, Guru Gobind Singh Indraprastha University, Delhi, India; ³Department of Urology, All India Institute of Medical Sciences, Delhi, India
(Presented By: Jyoti Sharma MTech)

Poster# 85
THE ASSOCIATION OF SEXUAL SATISFACTION WITH RECEIPT OF MEDICAL AND SURGICAL TREATMENT AMONG TRANSGENDER PATIENTS
R. Craig Sineath MPH¹, Joseph Gerth², Vin Tangpricha MD, PhD¹ and Michael Goodman MD, MPH²
¹Emory University School of Medicine; ²Emory University Rollins School of Public Health
(Presented By: R. Craig Sineath MPH)

Poster# 86
TESTOSTERONE, VITAMIN D, NUTRITIONAL AND METABOLIC STATUS. IS THERE A LINK AMONG THEN?
Inari Ciccone MSc Student¹, Juliana Pariz MD, PH¹, Elaine Costa MD, PHD¹ and Jorge Hallak MD, PHD¹
¹Department of Urology. University of Sao Paulo. Brazil/ Androscience — High Complexity Clinical and Research Andrology Laboratory. Brazil; ²Department of Endocrinology. University of Sao Paulo. Brazil/ Androscience — High Complexity Clinical and Research Andrology Laboratory. Brazil; ³Department of Urology. University of Sao Paulo. Brazil/ Androscience — High Complexity Clinical and Research Andrology Laboratory. Brazil/ CellOxess LLC
(Presented By: Inari Ciccone MSc Student)

Poster# 87
THE ASSOCIATION OF A HISTORY OF SEXUALLY TRANSMITTED INFECTIONS WITH SEMEN ANALYSIS PARAMETERS IN THE INFERTILE PATIENT
Nahid Punjani MD, MPH¹, Madhur Nayan MD, CM, PhD², Ethan Grober MD, MEd, FRCSC², Kirk Lo MD, CM, FRCSC², Susan Lau BSc² and Keith Jarvi MD, FRCSC²
¹Division of Urology, Western University, London, ON, Canada; ²Division of Urology, University of Toronto, Toronto, ON, Canada
(Presented By: Nahid Punjani MD, MPH)
POSTER SESSION III

Poster# 88
CORRELATING THE ANDROGEN DEFICIENCY OF THE AGING MALE (ADAM) SCORE TO SEMEN ANALYSIS IN THE INFERTILE PATIENT
Nahid Punjani MD, MPH¹, Madhur Nayan MD, CM, PhD², Ethan Grober MD, MEd, FRCSC², Kirk Lo MD, CM, FRCSC², Susan Lau BSc² and Keith Jarvi MD, FRCSC²
¹Division of Urology, Western University, London, ON, Canada; ²Division of Urology, University of Toronto
(Presented By: Nahid Punjani MD, MPH)

Poster# 89 - WITHDRAWN

Poster# 90
IS THERE A RELATIONSHIP BETWEEN INCREASING PATERNAL AGE, SPERM DNA DAMAGE, AND OXIDATIVE STRESS IN INFERTILE MEN?
Andrew Lee BSc¹, Bill Yee BSc¹, Annie Qu BSc¹, Sahib Shahani PhD¹, Sergey Moskovtsev MD, PhD¹, 4 and Clifford Librach MD¹, 5, 6, 7
¹ICReAte Fertility Centre, Toronto, ON, Canada; ²Department of Physiology, University of Toronto, ON, Canada; ³Department of Medicine, University of Toronto, ON, Canada; 4Department of Obstetrics and Gynecology, University of Toronto, ON, Canada; 5Department of Physiology; 6Department of Obstetrics and Gynecology, 5Institute of Medical Sciences, University of Toronto, ON, Canada; 76Department of Gynæcology, Women's College Hospital, Toronto, ON, Canada.
(Presented By: ANDREW LEE BSc)

Poster# 91
INDUCED PLURIPOTENT STEM CELL-DERIVED CONDITIONAL MEDIUM PROMOTES LEYDIG CELL ANTI-APOPTOSIS AND PROLIFERATION VIA AUTOPHAGY AND WNT/β-CATENIN PATHWAY
Xiaoling Guo Assistant Professor, Yong Chen Master¹, Tingting Hong Master², Xianwu Chen Master³, Yue Duan Master4, Chao Li professor5 and Renshan Ge professor6
¹15167795209; ²18858736027; ³18267857001; ⁴415990704936; ⁵513587663736; ⁶615258456828
(Presented By: Xiaoling Guo Assistant Professor)

Poster# 92
TIME SPENT WALKING AND SITTING: IS THERE AN ASSOCIATION WITH SEMEN PARAMETERS?
Justin Houman MD¹, Howard Kim MD¹ and Wendie Robbins PhD²
¹Cedars-Sinai Medical Center; ²University of California, Los Angeles
(Presented By: Justin Houman MD)

Poster# 93
CLASSICAL VERSUS NON-CLASSICAL HYPOGONADISM
Noah Lupica¹, David Guo MD², Katherine Rotker MD² and Kathleen Hwang MD²
¹Alpert Medical School of Brown University; ²Department of Urology, Alpert Medical School of Brown University; ³Department of Urology, University of Massachusetts Medical School
(Presented By: Noah Lupica)

Poster# 94
EFFECTS OF CROSS-SEX HORMONE THERAPY ON TESTICULAR MORPHOLOGY IN TRANS WOMEN AT TRANSSEXUAL PROGRAM IN A REFERRAL CENTER FOR ENDOCRINOLOGY IN BRAZIL
Ana Alice Maciel MD¹, Ana Clemente MD¹, Joao Madeira MD¹, Flavia Cunha PhD¹, Rafael Block MD¹, Natalia Lisboa MD¹, Berenice Mendonca MD, PhD, Prof¹, Sorahia Domenice MD, PhD and Elaine Costa MD, PhD, Prof¹
¹Division of Clinical Endocrinology, Hospital das Clínicas, Department of Endocrinology of University of São Paulo School of Medicine, São Paulo, Brazil; ²Division of Clinical Endocrinology, Hospital das Clínicas, Department of Endocrinology of University of São Paulo School of Medicine, São Paulo, Brazil
(Presented By: Elaine Costa MD, PhD, Prof)

Poster# 95
ONCOSTATIN M INHIBITS DIFFERENTIATION OF RAT STEM LEYDIG CELLS IN VIVO AND IN VITRO
Renshan Ge MD¹, Yiyian Wang MD², Lubin Xie MD² and Qingquan Lian PhD³
¹The Second Affiliated Hospital of Wenzhou Medical University; ²The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Renshan Ge MD)

Poster# 96
COMBINED USE OF GM1 LOCALIZATION PATTERNS AND SEMEN ANALYSIS PARAMETERS TO IDENTIFY INDIVIDUALS WITH HIGHER OR LOWER CHANGES OF ACHIEVING PREGNANCY
Eric Seaman MD¹ and Jay Schinfeld MD, FACOG²
¹New Jersey Urology; ²Abington Reproductive Medicine
(Presented By: Eric Seaman MD)
POSTER SESSION III

Poster# 97
INCREASED RISK OF CANCER IN MEN WITH PEYRONIE’S DISEASE
Taylor Kohn MPhil¹, Alexander Pastuszak MD PhD², Michael Eisenberg MD³, Dolores Lamb PhD² and Larry Lipshultz MD³
¹Baylor College of Medicine; ²Center for Reproductive Medicine & Scott Department of Urology, Baylor College of Medicine; ³Departments of Urology and Obstetrics/Gynecology, Stanford University School of Medicine
(Presented By: Taylor Kohn MPhil)

Poster# 98
IMPROVING POST-THAW SPERM CRYOSURVIVAL RATES IN THE ANDROLOGY LAB: CHOOSING THE BEST PROCESSING TECHNIQUE PREVIOUS TO THE CRYOPRESERVATION IN ACCORDING TO INITIAL SPERM CHARACTERISTICS
Juliana Pariz PhD¹,²,³,4, Beatriz de Campos BSc¹,5, Rosa Alice Monteiro BSc² and Jorge Hallak MD, PhD¹,³,6
¹Androscience—Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Lab, Brazil; ²Methodist University of Sao Paulo, Brazil; ³Dept. of Urology, Universidade de São Paulo (USP), Brazil; 4Reproductive Toxicology Unit, USP, Brazil.; 5Methodist University of Sao Paulo, Brazil.; 6Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil.
(Presented By: Adetunji Fayomi DVM, MVSc)

Poster# 99
LONG-TERM TESTOSTERONE THERAPY (TTH) WITH TESTOSTERONE UNDECANOATE INJECTIONS (TU) COMPLETELY PREVENTS PROGRESSION FROM PREDIABETES TO TYPE 2 DIABETES (T2DM) IN 45 HYPOGONADAL MEN WITH PREDIABETES: REAL-LIFE DATA FROM A REGISTRY STUDY
Farid Saad DVM, PhD¹,², Ahmad Haider MD, PhD³, Karim Haider⁴, Gheorghe Doros PhD⁴ and Abdulmaged Traish PhD⁵
¹Bayer AG, Berlin, Germany; ²Gulf Medical University, Ajman, UAE; ³Private Urology Practise; 4Boston University School of Public Health; 5Boston University School of Medicine
(Presented By: Farid Saad DVM, PhD)

Poster# 100
REDUCTION OF OBESITY, MORTALITY AND MAJOR ADVERSE CARDIOVASCULAR EVENTS (MACE) IN HYPOGONADAL MEN WITH TYPE 2 DIABETES (T2DM) RECEIVING LONG-TERM TESTOSTERONE THERAPY (TTH): REAL-LIFE DATA FROM A REGISTRY STUDY
Farid Saad DVM, PhD¹,², Ahmad Haider MD, PhD³, Karim Haider⁴, Gheorghe Doros PhD⁴ and Abdulmaged Traish PhD⁵
¹Bayer AG, Berlin, Germany; ²Gulf Medical University, Ajman, UAE; ³Private Urology Practise; 4Boston University School of Public Health; 5Boston University School of Medicine
(Presented By: Farid Saad DVM, PhD)

Poster# 101
SEmen QUALITY AND REPRODUCTIVE FUNCTION AS MARKERS OF GENERAL MALE HEALTH
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(Presented By: Alberto Ferlin MD, PhD)

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Betsy Cairo PhD, Lauren Goede, MS, and Juliana Tyo MS
CryoGam Colorado
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Karen Lockyear PhD³, Sergey Moskovstev PhD, MD³, Prati Sharma MD³, Ali Baratz MD³, Karen Glass MD³ and Clifford Librach MD³
³CREAtE Fertility Centre; ³CREAtE Fertility Centre, University of Toronto; ³CREAtE Fertility Centre, University of Toronto, Women’s College Hospital
(Presented By: Karen Lockyear PhD)

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(Presented By: Elaine Costa MD, PhD, Prof)
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National Cheng Kung University Hospital
(Presented By: Ta-Yao Tai MD)

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Shota Janjgava MD, PhD, Elene Giorgadze MD, PhD and Lasha Uchava MD, PhD
National Institute of Endocrinology
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Caroline Ranêa MSc student¹,²,³, Juliana Pariz PhD¹,²,³,4,5, Rosa Monteiro BSc student¹ and Jorge Hallak MD, PhD¹,²,3
¹Androscience – Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Lab, Brazil; ²Reproductive Toxicology Unit, USP, Brazil; ³Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil; 4Methodist University of Sao Paulo, Brazil; 5Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil.
(Presented By: Caroline Ranêa MSc student)

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Larissa Belardin BSc, MSc, Mariana Antoniassi BSc, MSc, Mariana Camargo BSc, MSc, PhD, Paula Intasqui BSc, MSc and Ricardo Bertolla DVM, PhD
Universidade Federal de São Paulo - UNIFESP
(Presented By: Larissa Belardin BSc, MSc)

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DETERMINATION OF HABITS AND LIFESTYLES THAT AFFECTS SPERM MOTILITY QUALITY IN MEN
Dayane Reis BSc¹,²,³, Juliana Pariz PhD¹,²,³,4, Victoria Coutinho BSc student¹,²,³,4, Caroline Ranêa MSc student¹,³, Inari Ciccone MSc student¹,³, Rosa Alice Monteiro BSc¹ and Jorge Hallak MD, PhD¹,²,3
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(Presented By: Dayane Reis BSc)

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Sabine Nguyen DO¹, Magda Shaheen MD¹, Senait Teklehaimanot PhD¹, Dulcie Kermah PhD¹, Monica Ferrini MS, PhD¹ and Jacob Rajfer MD²
¹Charles R. Drew University; ²UCLA
(Presented By: Jacob Rajfer MD)

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VASECTOMY REVERSAL UTILIZING FIBRIN GLUE REINFORCEMENT: ONE INSTITUTION’S EXPERIENCE
Graham Machen MD, Colin Kleinguetl MD, Wencong Chen PhD and Erin Bird MD, MBA
Scott and White Medical Center
(Presented By: Graham Machen MD)

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Ibukun Oyeyipo BSc, MSc, PhD, Michelle van der Linde BSc, MSc and Stefan du Plessis BSc, MSc, PhD, MBA
Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa.
(Presented By: Ibukun Oyeyipo BSc, MSc, PhD)

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Rosa Alice Monteiro BSc¹, Juliana Pariz PhD¹,²,³,4,5, Bruna Zillig BSc student¹,²,³,4,5, Donald Evenson PhD6 and Jorge Hallak MD, PhD¹,²,3
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(Presented By: Rosa Alice Monteiro BSc)
POSTER SESSION III

Poster# 114
CASE REPORT: VARICOCELECTOMY IMPROVES SEMEN QUALITY AND THE ABILITY OF SPERM TO UNDERGO CAPACITATION
Eric Seaman MD¹ and Samuel Aly MD²
¹New Jersey Urology; ²Rutgers - New Jersey Medical School
(Presented By: Eric Seaman MD)

Poster# 115
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Jianhua Guo
(Presented By: Jianhua Guo)

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Vidhu Dhawan MD¹, Manoj Kumar PhD¹, Dipika Deka MD², Neena Malhotra MD², Neeta Singh MD², Vatsla Dadhwal MD² and Rima Dada MD, PhD¹
¹Laboratory for Molecular Reproduction & Genetics, Dept. of Anatomy, AIIMS; ²Dept. of Obstetrics & Gynaecology, AIIMS
(Presented By: Vidhu Dhawan MD)

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Logan Nickels, Mitch Eddy PhD and David Sokal MD
Male Contraception Initiative
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ABSTRACTS

SUNDAY, APRIL 22, 2018
*Poster Session I
11:30 a.m. - 12:30 p.m.
Location: The Nines Ballroom PreFunction
*Not CME Accredited

1 (Oral/Poster)
CRISPR-CAS9 MEDIATED KNOCKDOWN OF ODF2 IN PORCINE TESTICULAR SOMATIC CELLS
Taylor Goldsmith BSc, Dennis Webster¹, Dan Carlson PhD² and Ina Dobrinski DVM, PhD²
¹Recombinetics Inc; ²University of Calgary
(Presented By: Taylor M. Goldsmith, BSc)

Most of the cells in the body possess non-motile primary cilia. They can act as sensors, mediate cell-to-cell communication, and are essential in development and for differentiation of stem cells. Primary cilia are present on somatic cells of the testis, with the number of cilia decreasing as the testes matures. This suggests that primary cilia are important in the morphogenesis of the testes. We hypothesize that ablation of primary cilia from testicular somatic cells will inhibit testicular morphogenesis. To test this, we targeted outer dense fiber protein 2 (ODF2), a protein essential for cilia formation, using CRISPR-Cas9 in porcine testicular somatic cells. We then investigated the effects of primary cilia ablation on tubular morphogenesis in vitro. Testicular somatic cells were isolated from one week old piglets through enzymatic digestion and separated from germ cells by differential plating. Two exons of Odf2 were targeted using the CRISPR-Cas9 system with Odf2 guide RNA delivered using nucleofection. Cilia loss was assessed by immunofluorescence for ARL13B, a ciliary GTPase. Cells transfected with a CRISPR construct lacking the guide RNA sequence served as controls. To study tubular morphogenesis in vitro, cells were cultured on Matrigel and analyzed for tubule formation by immunofluorescence. Treatment with CRISPR-Cas9 caused a significant reduction in the number of primary cilia on testicular somatic cells (p<0.0001). In targeted cells, 23.8% +/- 6.26 of cells had cilia, compared to 68.74% +/- 6.26 unmodified cells with cilia (n=4). Quantitative PCR confirmed knockdown of Odf2 expression in cells that were transfected with the CRISPR constructs, with 5.6-fold lower expression of Odf2 compared to controls (n=2). When transfected cells were used to form tubules in vitro, cells where Odf2 had been targeted formed thinner, shorter, and less organized tubules compared to tubules formed from sham edited controls. These results indicate that primary cilia play a role in tubular morphogenesis in vitro and this approach will provide the basis for elucidating signaling pathways involved in tubule formation.

Supported by NIH/ORIP 9 R01 OD016575-12

2 (Oral/Poster)
INVESTIGATING ZIKA VIRUS PATHOGENESIS ON MALE REPRODUCTION USING A HUMAN 3-DIMENSIONAL TESTICULAR ORGANOID MODEL
Nima Pourhabibi Zarandi MD¹, Daniel Strange², Goral Trivedi², Anthony Atala MD³, Colin Bishop PhD³, Saguna Verma PhD³ and Hooman Sadri-Ardekan MD PhD²
¹Wake Forest Institute for Regenerative Medicine and Department of Urology, Wake Forest School of Medicine, Winston Salem, NC; ²Department of Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI
(Presented By: Nima P. Zarandi, MD)

Introduction: An increase in the number of newborns with microcephaly in women infected with Zika virus (ZIKV) in the 2015-2016 global epidemic coincided with the detection of ZIKV in the semen. This raised concerns regarding the sexual transmission of this primarily mosquito-transmitted virus. Recent reports of detecting ZIKV in seminal fluid months after clearance of the viremia in as high as 56% of infected men, together with a low sperm count, suggested the ability of ZIKV to enter into the immune privileged compartment of the testes and establish persistent infection. The lack of any specific therapeutic drugs to clear testicular infection and the absence of any appropriate animal model to mimic human testicular ZIKV infection has brought an urgent need for the development of human in vitro systems to understand the associated mechanisms of viral persistence.

Objective: Utilize a 3D human testicular organoid system to support robust infection of ZIKV as a novel model system to assess the cell tropism of the virus and infected testicular cell survival.

Methods: Cryopreserved, multicellular 3D human testis organoids (HTOs) constructed from cultured adult human testicular cells were thawed and used after a 5-day recovery period. HTOs were infected with 105 PFU of ZIKV (PRVABC59) for 1 hour at 37°C. Twenty four and 72 hours after infection RNA virus copy number, plaque assay, ATP activity and cells specific gene expression were compared between control and infected HTOs.

Results: Low copies of ZIKV RNA were detected at 24hrs that increased significantly by more than 2 logs at 72 hours. A similar trend was observed in the plaque assay with a significant increase in infectious virions at the 72hr time point. ATP production showed a dramatic decrease after 72hrs that is comparable with the peak virus copy numbers. Expression levels of ZBTB16 (Spermatogonial cell marker), CYP19A1 (Sertoli Cell marker) and STAR (Leydig cell marker) were down regulated significantly after 72 hours.

Conclusion: This preliminary study provides evidence that ZIKV can efficiently infect HTOs and affect different types of testicular cells. Future research using this 3D HTO system will focus on long-term replication and persistence of ZIKV in the testes. This may open exciting new avenues for basic and translational research on testes-tropic viruses including testing efficacy and toxicity of anti-virus drugs in clearing testicular infection.

3 (Oral/Poster)
IN VITRO CULTURE OF HUMAN KLINEFELTER SPERMATOGONIAL STEM CELLS
Guillermo Galdon MD¹, Nima Pourhabibi-Zarandi MD¹, Mark Pettenati PhD², Stuart Howards MD¹, Stanley Kogan MD FACS³, Anthony Atala MD³, and Hooman Sadri-Ardekan MD PhD²
¹Wake Forest Institute for Regenerative Medicine; ²Section of Medical Genetics, Department of Pathology Wake Forest School of Medicine; ³Department of Urology; ⁴Wake Forest School of Medicine; ⁵Wake Forest School of Medicine, Wake Forest Institute for Regenerative Medicine
(Presented By: Guillermo G. Lopez, MD)

Introduction: Klinefelter Syndrome (KS) is characterized by masculine phenotype, supernumerary sex chromosomes (47 XXY) and impaired fertility due to progressive loss of spermatogonial stem cells (SSC) starting at the onset of puberty. It’s been hypothesized that early SSC cryopreservation could be an option for future fertility treatments in these patients including SSC transplantation. Establishment of an in vitro culture system is critical to preserve future fertility in KS patients.

Objective: Isolate and propagate human KS spermatogonia stem cell from cryopreserved tissue.

Methods: Testicular tissue from KS patients enrolled in experimental testicular tissue bank at Wake Forest Baptist Health has been cryopreserved. Testicular cells were isolated and propagated in long term culture. Propagated testicular cells were characterized using q-RTPCR, digital-RT PCR, Flow Cytometry and MACS-sorted X/Y chromosomes FISH staining.

Results: Growing KS human testicular cells from 3 non-mosaic KS patients (13, 15 and 17 years old) showed viable testicular cells up to 120 days in culture. During this period, cells were expanded more than 2-million-fold. Cell specific gene expression revealed the presence of all 4 testicular cell types including spermatogonia, Sertoli, Leydig and Peritubular cells. A population of ZBTB16+ undifferentiated spermatogonia (25% of all cultured cells) was identified all along culture using digital PCR. Flow Cytometry analysis detected a HLA/-CD9+/-CD49f+ population (8% of all cultured cells) suggesting a...
stem cell subpopulation. FISH staining for chromosomes X and Y on CD9+ MACS sorted spermatogonia confirmed KS sexual trisomy along the culture. Interestingly, using serial FISH detected a population of cells losing the extra X chromosomes in later passages (Figure 1).

**Figure 1:** FISH images of XXY testicular cells in culture. Orange probe stains X chromosome and Green probe stains Y chromosome specifically. XY Spermatogonial (Arrow). Scale bar 10 μm.

**Conclusion:** To our best knowledge this is the first report of successful isolation and propagation of testicular cells from human KS patients. This culture system has potential for either in vitro or in vivo fertility preservation in KS patients.

**4 (Oral/Poster)**

**PREGNANCY ESTABLISHED AFTER AUTOLOGOUS GRAFTING OF CRYOPRESERVED TESTICULAR TISSUE FROM PREPUBERTAL RHESUS MACAQUES**

Adetunji Fayomi DVM, MVSc¹, Peters Karen BS², Meena Sukhwani PhD³, Cathy Ramsey BS³, Carol Hanna PhD³, Jon Hennebold PhD² and Kyle Orwig PhD³

¹Magee-Womens Research Institute, University of Pittsburgh School of Medicine; ²Magee-Womens Reserarch Institute University of Pittsburgh School of Medicine, Pittsburgh, PA; ³Assisted Reproductive Technology Core, Oregon National Primate Research Center, Beaverton, OR

(Presented By: Adetunji Fayomi, DVM, MVSc)

**Introduction and Objective:** Testicular tissue freezing is an experimental fertility preservation option for prepubertal boys who are not able to cryopreserve sperm. The objective of this study was to demonstrate that fertilization competent sperm could be recovered from frozen/thawed grafts from prepubertal Rhesus macaques.

**Materials and Methods:** Testicular tissue of five prepubertal Rhesus macaques was collected and cut into small pieces (2-5 mm³). Fresh or frozen/thawed tissues were grafted under the back skin (3 fresh & 3 frozen) and in the scrotum (1 fresh & 1 frozen). Matrigel was introduced into four sites on the back and both scrotal sites. Grafts were recovered for analysis after 8-10 months. Graft volume, follicle stimulating hormone (FSH) and testosterone (T) levels were monitored throughout the grafting period. At the end of the study, recovered grafts were weighed and fixed for immunofluorescence (IF; VASA, ACROSIN, GFRA1) or hematoxylin/eosin (HE) staining. Portions of each graft were teased apart and/or digested with collagenase to release sperm that were used to fertilize Rhesus eggs by intracytoplasmic sperm injection (ICSI). Quantitative results were expressed as mean ± standard error of the mean, and paired sample T-test was used to identify differences between treatment groups (p<0.05).

**Results:** HE and IF confirmed that donor tissues were immature (no spermatogenesis) at the time of grafting. Irrespective of the graft site or matrigel treatment, graft volume increased over time in the frozen/thawed as well as fresh grafts. FSH and T levels increased after grafting, indicating progression through puberty and an intact hypothalamic, pituitary, gonadal axis. Graft weights from the scrotum were higher than from the back (P<0.01), but complete spermatogenesis was confirmed in all grafts with spermatids in more than 70% of seminiferous tubules. Irrespective of experimental group, millions of sperm were recovered per graft and sperm were competent to fertilize Rhesus oocytes, leading to preimplantation embryo development up to the hatching blastocyst stage in vitro. Embryos were transferred into surrogate Rhesus females which results in pregnancy. Evaluation of fetal development is ongoing.

**Conclusion:** Testicular tissue grafting is a mature technology that may be ready for the human fertility clinic.

**Support:** This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development grant HD075795.

**5 (Oral/Poster)**

**PUBERTAL DEVELOPMENT AFTER TESTICULAR TISSUE BIOPSY FOR FERTILITY PRESERVATION.**

Aude Braye PhD-student¹, Inge Gies Prof Dr², Veerle Vloeberghs Dr², Alina Ferster Prof Dr³, Jutte Van der Werff Ten Bosch Prof Dr², Herman Tournaye Prof Dr² and Ellen Goossens Prof Dr³

¹Vrije Universiteit Brussel; ²Universitair Ziekenhuis Brussel; ³Hôpital Universitaire Des Enfants Reine Fabiola

(Presented By: Aude Braye, Master)

**Introduction:** Male infertility and the search for an adequate fertility preservation strategy becomes clinically more relevant because of the improved survival rate of childhood cancer patients and the earlier diagnosis of Klinefelter Syndrome (KS) patients due to better diagnostic methods. Currently, the only fertility preservation option for these young boys is undergoing a testicular tissue biopsy followed by testicular tissue banking. Although recent evidence demonstrates that the immediate adverse effects of the biopsy procedure are rare (1%), nothing is known on its possible adverse effects on the long-term. The aim of this project is to investigate how a testicular tissue biopsy procedure at young age may affect the pubertal development of boys.

**Methods:** All paediatric patients needing gonadotoxic treatment or patients with KS seen at the Universitair Ziekenhuis Brussel between 2002 and 2016 were enrolled in this cohort study. Hormone serum levels important during pubertal development, testicular volume, bone age and bone density were recorded at different time points over several years and compared between patients who underwent a testicular tissue biopsy and those who did not. Data were analysed using the Chi-squared statistical test.

**Results:** 113 boys needing gonadotoxic treatment and 109 KS patients were enrolled in this retrospective cohort. The testes of 63 boys needing gonadotoxic treatment (56%) and the testes of 37 KS patients (34%) were biopsied. No differences were observed for luteinizing hormone, follicle-stimulating hormone, testosterone, inhibin-B, testicular volume and bone age between patients who did and those who did not undergo a testicular tissue biopsy. For anti-müllerian hormone, estradiol and bone density, not enough data were available to make any Conclusion. We also observed that the need for substitution treatment occurred at the same Tanner stage for patients who did and those who did not undergo a testicular tissue biopsy.

**Conclusion:** These results demonstrate that a testicular tissue biopsy at young age has no additional adverse effect on the pubertal development of young boys. The large number of missing data proves the need to establish a more standardized follow-up protocol for boys undergoing testicular tissue biopsy at young age.

**6 (Oral/Poster)**

**EPIDIDYMAL SPERM MATURATION – ROLES OF CALCINEURIN, PP1G2, AND GSK3**

Souvik Dey PhD¹, Alaa Eisa MS², Suranjana Goswami MS¹, Rahul Bhattacharjee MS¹, Douglas Kline PhD¹ and Srinivasan Vijayaraghavan PhD¹

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(Presented By: Souvik Dey, Postdoc/PhD)

In mammals, initiation of motility and acquisition of the ability to fertilize eggs develop during passage of sperm through the epididymis. The mechanistic details of the biochemical basis for epididymal sperm maturation are not known. Changes in cAMP action and protein phosphorylation were thought to be involved. Two new discoveries should enable us to understand the biochemical basis underlying epididymal sperm maturation. One of the signaling proteins identified...
by gene deletion to play a role in epididymal sperm maturation is the calcium regulated protein phosphatase calcineurin. Another signaling protein is glycogen synthase kinase 3 (GSK3). We identified GSK3 as the regulator of the sperm-specific protein phosphatase. The only phenotype of mice with global or selective knock out of Gsk3a in developing spermatocytes/spermatids is male infertility. Caudal epididymal sperm lacking GSK3a resemble wild type immature caput epididymal sperm. The purpose of this study was to examine the interrelationships between calcineurin, PP1g2, and GSK3a in acquisition of motility and fertilizing ability during their passage through the epididymis.

We show that both calcineurin and PP1g2 affect GSK3α activity. We have verified earlier data that immature epididymal sperm contain significantly higher calcium levels compared to mature caudal epididymal sperm suggesting that an active calcineurin is essential during sperm maturation. We determined that the association of the regulators of the catalytic activity of PP1g2, inhibitors I2 and I3 and sds22, change during epididymal sperm maturation. Changes in the association of the regulators with PP1g2 are impaired in sperm lacking GSK3a resulting in reduced ATP generation through glycolysis. Surprisingly, sperm from calcineurin knock out mice have higher ATP levels compared to wild type sperm. Lack of calcineurin leads to an abnormal increase in mitochondrial potential and ATP production that is not synchronized with development of sperm motility and fertility potential. Sperm lacking GSK3 or calcineurin are also unable to fertilize eggs in vitro. Wild type sperm treated with calcineurin or GSK3 inhibators reduce in vitro fertilization of eggs.Taken together our data show that calcineurin and GSK3a are the two missing pieces essential to the understanding the biochemical basis underlying activation of metabolism that accompanies sperm motility and fertility development in the epididymis. Supported by (NIH HD086839).

7 (Oral/Poster)
ABSENCE OF 14−3−3 EPSILON ALTERS MALE FERTILITY, SPERM COUNT, AND MOTILITY IN MICE
Alaa Eisa¹, Alexander Ignatious², Souvik Dey², Sumit Bhutada², Srinivasan Vijayaraghavan² and Douglas Klime²
¹School of Biomedical Science, Kent State University; ²Department of Biological Sciences, Kent State University
(Presented By: Alaa Eisa)

Spermatogenesis requires a number of complex processes that regulate meiosis and cellular differentiation. Spermatogenesis involves the synthesis of new proteins and the complex activation and inhibition of proteins, particularly those involved in cellular signaling. Many cellular processes are regulated by the phosphorylation status of the protein. In addition to the phosphatases and kinases that play a major role in activation and suppression proteins by phosphorylation, other proteins, such as 14−3−3 proteins, play role in coordinating the activity of these kinases and phosphatases. The 14−3−3 (YWHA) proteins are known to be key regulatory proteins in many cellular processes including the regulation of the cell cycle during meiosis. There are seven isoforms of the 14−3−3 that encoded by different seven genes (Ywhab, Ywhae, Ywhag, Ywhaa, Ywhah, Ywhaz and Ywhas). It is known that 14−3−3 proteins are expressed in testis and sperm; however, the role for each of the seven isoforms has not yet been fully characterized. The role of 14−3−3 epsilon was studied in this project by using a testis-specific conditional knockout (KO). These mice contain LoxP sites to remove exons 3 and 4 of 14−3−3 epsilon by using monoclonal antibodies against the 14−3−3 epsilon. The sperm count was significantly lower compared to the control heterozygous LoxP+/− (cre−/−) littermates. Using the Computer Assisted Semen Analysis (CASA) system, the total and progressive motility of the 14−3−3 epsilon KO sperm was significantly lower compared to the control sperm. Preliminary data indicates a decrease in phosphorylated serine and tyrosine residues in Glycogen Synthase Kinase 3 (GSK3), and an alteration in the phosphorylation status of Protein Phosphatase 1 (PP1) in 14−3−3 epsilon KO mice, suggesting the absence of 14−3−3 epsilon KO may alter signaling pathways known to regulate sperm motility.

8 (Oral/Poster)
A MOUSE GOLGIN PROTEIN, GAMP-210 IS REQUIRED FOR ACROSOME FORMATION BUT NOT FLAGELLA FORMATION
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(Presented By: Suheng Ma)

The golgin GMAP-210, also known as Thyroid Hormone Receptor Interactor 11 (TRIP11), is a cis-Golgi network-associated protein. The protein is required for efficient trafficking in the secretory pathway. Mice lacking GMAP-210 die at birth with a pleiotropic phenotype that includes growth restriction, ventricular septal defects of the heart, omphalocele, and lung hypoplasia. It has been shown that GMAP-210 determines IFT20, a subunit of intraflagellar transport complex localization in the Golgi bodies. Cells lacking GMAP210 have normal Golgi structure, but IFT20 is no longer localized to this organelle. Our laboratory discovered that IFT20 is essential for male fertility and spermiogenesis in mice. To investigate the role of GMAP-210 in male fertility and spermatogenesis, the floxed Gmap-210 mice were bred with Stra8−Cre mice so that the Gmap-210 gene is disrupted in spermatocytes/spermatids. The Gmap-210:Stra8−Cre mutant mice did not show any gross abnormalities, and all of the mutant mice survive to adulthood. There was no difference in testis weight/body weight between controls and mutant mice. Histological examination of the testes revealed normal seminiferous tubule structure. Sperm number was slightly reduced compared to the controls. All adult homozygous mutant males examined had significantly reduced sperm motility. Sperm flagella appear to be normal as examined by light microscopy; however, almost all the heads were abnormally shaped. In the control mice, IFT20 was present in the Golgi bodies of spermatocytes, the acrosome, and manchette of spermatids. In the conditional Gmap-210 knockout mice, IFT20 was not present in these localizations; instead, IFT20 signal was scattered throughout the cytoplasm. An acrosome maker, peanut-lectin was almost absent in the spermatids of the conditional Gmap-210 knockout mouse. Collectively, our findings suggest that GMAP-210 is essential for acrosome formation and sperm motility. Even though GMAP-210 regulates IFT20 localization in male germ cells, and IFT20 is required for normal spermiogenesis and sperm flagella formation, other mechanisms might compensate for normal sperm flagella formation in the absence of GMAP-210 in male germ cells.
9 (Oral/Poster)  
**NEW INSIGHTS INTO THE VITAMIN D CONCENTRATION AND ITS ASSOCIATION WITH SEMEN QUALITY IN MALE SUBJECTS**  
Inari Ciccone MSc Student¹, Juliana Pariz MD, PH¹, Elaine Costa MD, PH², Parviz Gharagozloo MD, PH³, John Atiken PHD⁴ and Jorge Hallak PHD⁵  
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(Presented By: Inari Ciccone, Msc Student)  

**Background:** Vitamin D is a versatile signaling molecule, that targets also male reproductive organs, in addition to the classic effects on bone, calcium and phosphate homeostasis. Accumulating evidences from animal and human studies suggest that vitamin D is involved in reproduction functions in both genders. Objective: To evaluate the vitamin D relationship with semen quality in male with seminal parameters alteration and normozoospermic diagnosis.  

**Patients and Methods:** We selected 260 men (aged 18 to 60 y.o.) from a private andrology reference medical clinic. They were divided in two groups: Group 1: Normal seminal parameters (n=124) and Group 2: Abnormal seminal parameters (n=136). 25(OH) vitamin D serum concentration and lifestyle data were collected. Semen was analyzed according to WHO 2010 guidelines, PH, volume, motility, concentration, morphology, strict criteria and sperm functional tests were performed (ROS, CK, beads). Additionally, karyotype, frequency of varicocele, smoking, alcohol ingestion, and body composition were considered. Statistical analysis was performed by SPSS program version 19.0 (SPSS Inc., Chicago, IL). Spearman correlation, Mann-Whitney test and regression model were applied. Statistical significance was considered with P value < 0.05.  

**Results:** The mean 25(OH)D concentration were significantly lower in Group 2 (p=0.016) and all seminal parameters had a positive correlation with 25(OH)D serum levels. The highest correlation coefficient value was observed on the association of total motility with Vitamin D (p=0.001). No significance influence was observed among the lifestyle data, body composition and varicocele with seminal parameters. All patients had normal karyotype.  

**Conclusion:** Our results demonstrated that 25(OH)D levels has a positive influence on spermatogenesis and semen quality, suggesting that vitamin D replacement should highly be considered on male fertility treatment once low vitamin D levels have reached epidemic proportions in industrialized cities like Sao Paulo.  

11 (Poster)  
**GEMINI STUDY: FINE-MAPPING THE GENETIC ARCHITECTURE OF SEVERE MALE INFERTILITY**  
Liina Nagirmai¹, Jannette Rusch¹, Alexandre Lopes¹,², Kristian Almstrup¹, Brendan Houston¹, Ewa Rajpert-De Meyts¹, Marius Laan¹, Moira O’Bryan¹, Kenneth I. Aston³, Donald F. Conrad¹ and the GEMINI Consortium  
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(Presented By: Liina Nagirmai, PhD)  

**Introduction:** Male infertility due to spermatogenic failure is a common disorder found in 30 million men worldwide. Genetic factors are known to contribute to the manifestation of the disease, however the properties of the underlying genetic variation have largely remained elusive. It is feasible that rare patient-specific mutations in genes essential for sperm development may lead to the disease. An international network of andrologists and geneticists have established a multi-center study Genetics of Male Infertility Initiative (GEMINI) to fine-map the genetic landscape of idiopathic severe male infertility.  

**Objectives:** We aimed to perform whole exome sequencing and case-by-case mutation discovery in a large cohort of non-obstructive azoospermia (NOA) cases to identify rare variants of large effect as the likely disease cause.  

**Methods:** Whole exome sequencing data was generated on 890 well phenotyped NOA cases from 6 countries. Exome libraries were sequenced on Illumina HiSeq2500/3000 and analyzed using bwa, Picard and GATK tools. Case-by-case variant prioritization and annotation was performed using PSAP and in-house pipelines. Genes of interest were functionally validated by experimental manipulation in model organisms, mainly Drosophila melanogaster.  

**Results:** Putative rare disease-causing genetic variants were identified in 44% of patients. The affected genes were largely case-specific (92%) and previously undescribed in NOA. The largest fraction of cases (45%) were characterized by autosomal dominant mutations, half of which were loss-
Intraflagellar transport protein 20 (IFT20) is an essential factor for the control of spermatogenesis and male fertility in mice. To explore the mechanism of how IFT20 regulates spermatogenesis, a yeast two-hybrid screening was carried out with the full-length mouse IFT20 protein as a bait, and COP9 was identified to be a potential major binding partner. In mice, COSP5 is highly abundant in the testis, and it is also expressed in other tissues including the spleen and brain, but not in the kidney. During the first wave of spermatogenesis, COSP5 is detectable from day eight after birth; its level was dramatically increased at day 22 after birth; and the high level was maintained until day 35 after birth. Immunofluorescence staining on a normal adult mouse testis demonstrated that COP9 signal was highly concentrated in the acrosome. To investigate the role of COP9 in male fertility and spermatogenesis, the floxed Cops5 mice were bred with Stra8−iCre mice so that the Cops5 gene is disrupted specifically in male germ cells. The Cops5lox/lox: Stra8−iCre mutant mice survived to adulthood, but none of them were fertile. Sperm number and motility were dramatically reduced compared to the controls. Testis size was dramatically reduced in these adult mice as well. Histological examination of the testes revealed abnormal seminiferous tubule structure. Examination of the seminiferous tubules of the adult mice by transmission electronic microscopy revealed dramatically increased apoptotic cells at pre-meiotic stage, and this was confirmed by the histologic examination of testes during the first wave of spermatogenesis. An acrosome maker peanut-lectin was almost absent in the surviving spermatids. In the conditional Ift20 mutant mice, COPS5 localization and testicular expression levels were not changed. IFT20 expression level was not altered in the conditional Cops5 knockout mice. COP9 has been shown to be involved in multiple signal pathways, particularly functions as a co-factor to inhibit apoptosis. COP9 is believed to maintain normal spermatogenesis through multiple mechanisms, including maintaining male germ cell survival and acrosome biogenesis.
ABSTRACTS

16 (Poster)

SPERM PRODUCTION IN DE NOVO FORMED SEMINIFEROUS TUBULES FROM ALLOGENEIC TRANSPANTAL TESTICULAR CELLS IN RHESUS MONKEY TESTIS

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(Presented By: Gunapala Shetty, PhD)

To preserve fertility in prepubertal males, tissue or cell suspensions containing spermatogonial stem cells (SSCs) are currently being cryopreserved to eventually using them for the production of sperm. In such cases although SSC transplantation into the seminiferous tubules seems to be a good option, alternative approaches may be needed since therapeutic exposures can also cause tubular somatic cell damage in prepubertal testes rendering the tubules unsuitable for the development of donor SSCs. Previously de novo seminiferous tubule formation from testicular cells of mice and farm animals, with sperm in few cases, has been demonstrated after xenografting in recipient mice. Here we examined whether a similar phenomenon can occur in non-human primates.

A pubertal recipient monkey irradiated with 7 Gy, was treated with GnRH-antagonist, as that improves transplantation. Then cryopreserved testicular cells from a prepubertal monkey were transplanted to one of the testes. Since the transplantation was allogenic, the recipient was immunosuppressed with anti-CD154. Nine months after transplantation, the untransplanted testes showed differentiated germ cells in only 3% of the tubules. However in the transplanted testis, surprisingly, 20% of the volume consisted of irregularly shaped abnormal seminiferous tubules, 89% of which had differentiating germ cells, including testicular sperm. Some areas of inflammation were also noted. DNA microsatellite analysis of tissues collected by Laser Capture Microdissection from the testis sections showed the following percentages of the donor-genotype in tissues from regions of the testis: inflammatory, 0%; normal tubule region, 0%; abnormal tubule region, 66%; cells from the whole interior of abnormal tubules, 97%; and cells from the adluminal region of the abnormal tubules, 92%. Thus these abnormal tubules, including the enclosed germ cells were derived de novo from the donor testicular cells. Furthermore, the percentage of endogenous tubules containing differentiating germ cells was higher adjacent to the de novo tubules than further away, suggesting that these tubules support differentiation in the neighboring endogenous tubules by a paracrine mechanism. Thus de novo tubules formed from transplanted somatic cells can support differentiation of transplanted SSCs into sperm and represent a new and promising strategy for the restoration of fertility in male childhood cancer survivors.

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17 (Poster)

IN VIVO AND IN VITRO EFFECTS OF THE ENDOCRINE DISRUPTORS GENISTEIN AND MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) ON RODENT MACROPHAGE INFLAMMATORY RESPONSES

Vanessa Brouard PhD¹, Shahrzad Ghazisaeidi Master², Berenice Collet Master³, Annie Boisvert Master² and Martine Culty PhD²

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(Presented By: Vanessa Brouard, PhD)

Experimental and epidemiological evidence support the hypothesis of “Testicular dysgenesis syndrome”, proposing that perinatal exposure to endocrine disruptor chemicals (EDCs) disrupts the male reproductive system. We previously found that fetal exposure to a mixture of the plasticizer di-(2-ethylhexyl) phthalate (DEHP) and the phytoestrogen genistein (GEN) at a dose relevant to humans altered testis morphology, transcriptome and function, in neonatal and adult rats, suggesting the
ABSTRACTS

Involvement of inflammatory processes. The goal of this study was to examine the potential role of macrophages in the effects of GEN and DEHP, using both in vivo and in vitro approaches. In vivo studies: pregnant SD rats were gavaged with corn oil, 0.1 or 10 mg/kg/day of DEHP, GEN or the mixture, from gestation day 14 to birth. The testes of neonate and adult male offspring were collected and the mRNA expression of monocyte and macrophage-related genes was determined by gene array and quantitative real-time PCR (qPCR) analyses. Protein expression was studied by immunohistochemistry (IHC). In vitro studies were performed on macrophage cell lines, to examine whether GEN and MEHP, the bioactive metabolite of DEHP, at 10 or 100 μM, acted directly on basal and lipopolysaccharide (LPS)-induced macrophage activation. Fetal exposure led to changes in the expression of several macrophage-associated genes in neonatal and adult EDC-exposed testes. The tetraspanins CD63 and FcγR1a (CD64) were among the genes significantly increased only by the mixture in adult testis. Different effects were observed, depending of the genes, ages and doses. Among them, TNFa was increased in adult testis by the higher EDC dose, but decreased by the lower one. In vitro macrophage treatment with GEN and/or MEHP showed effects of the EDCs and their mixture on basal and LPS-activated gene expression. The chemokine Cxcl2 was increased by 3 hr treatment with GEN or MEHP, and their mixture potentiated the effect of LPS. By contrast, Il1b expression in basal and LPS-treated cells was decreased by 24 hr exposure to GEN and the mixture. These results suggest that fetal exposure GEN and DEHP promote inflammation in testis, and that GEN and MEHP directly alter macrophage functions, suggesting that some of the in vivo effects might arise from direct effects of GEN and DEHP on macrophages. These data also highlight the differences between single and mixed EDC effects. 

Work supported by funds from USC and a CIHR grant to MC

18 (Poster)
A NUCLEOCYTOPLASMIC TRANSPORT PROTEIN IMPLICATED IN BMP SIGNALING CONTROL IS REQUIRED FOR FERTILITY
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(Presented By: Katherine Loveland PhD)

Importin proteins bind and transport distinct sets of protein cargoes into the nucleus, hence their levels and activity control gene expression. We previously reported that the importin 5 protein (IPO5; previously RanBP5) is present in fetal germ cells of both testis and ovary, and moves between the nucleus and cytoplasm in a sex-specific manner. In postnatal spermatogenesis, IPO5 mRNA is highly upregulated in round spermatids and IPO5 protein is present in elongating spermatids and sperm. The tight regulation of IPO5 synthesis, and its high expression level in testis relative to other tissues, indicates it serves stage-specific roles vital for the cellular transitions of spermatogenesis. IPO5 is implicated in transport of proteins of relevance to the germline, Stella and Musashi 1, while more general roles in transporting factors to influence Wnt and BMP signaling have been identified. In studies with the TCam-2 seminoma cell line, which exhibits features of human gonocytes and responds differently to BMP4 and activin in terms of cell viability (Young et al, 2011, Int J Androl). Our preliminary results indicate that reduction of IPO5 by siRNA changes Smad nuclear accumulation in response to exposure to these ligands, potentially revealing a key role for IPO5 in germline differentiation and cell fate outcomes.

19 (Poster)
PIPERINE PROMOTES PUBERTAL LEYDIG CELL DEVELOPMENT BUT INHIBITS SPERMATOGENESIS IN RATS.
Xianwu Chen MD, Fei Ge MD, Qingquan Lian PhD and Ren-shan Ge PhD
The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University
(Presented By: Xianwu Chen, MD)

Background: Piperine is the primary pungent alkaloid isolated from black peppercorns of the fruit bodies of Piper nigrum. Piperine is used in dietary supplements and traditional medicine. The object of the present study was to investigate the effects of piperine on pubertal development of Leydig cells in the rat.

Methods: Piperine (0 or 5 or 10 mg/kg) was gavaged to 35-day-old male Sprague-Dawley rats for 30 days. Serum hormone (testosterone, luteinizing hormone, and follicle-stimulating hormone) levels were measured and Leydig cell numbers and size, gene and protein expression were investigated on day 65 postpartum. Immature Leydig cells were isolated from 35-day-old male rats and treated with 50 μM piperine for 24 h and androgen production in the presence of luteinizing hormone, 8bromo-cAMP, 22R-hydroxysterololesterol, pregnenolone, progesetorone, and androstenedione was measured.

Results: Piperine significantly increased serum testosterone level. Piperine reduced follicle-stimulating hormone level without affecting luteinizing hormone level. Piperine increased Leydig cell number and cell size, and upregulated steroidogenic acute regulatory protein, cholesterol side-chain cleavage enzyme, 3beta-hydroxysteroid dehydrogenase 1, 17 beta-hydroxylase/20-lyase, and steroidogenic factor 1 expression. Piperine in vivo inhibited spermatogenesis. Piperine in vitro also increased androgen production and stimulated cholesterol side-chain cleavage enzyme and 17 beta-hydroxylase/20-lyase activities in immature Leydig cells.

Conclusion: Piperine stimulates pubertal Leydig cell development by increasing Leydig cell number and promoting its maturation, but it inhibits spermatogenesis.

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20 (Poster)
HUMAN SPERMATOGONIAL STEM CELL CULTURE USING TISSUE-DERIVED EXTRACELLULAR MATRICES
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(Presented By: Sherin David)

Background: Pediatric cancer patients are susceptible to testicular tissue damage from exposure to cytotoxic chemotherapeutic agents and radiation. Fertility in these patients can potentially be restored using spermatogonial stem cell (SSC) transplantation, which requires the expansion of SSCs isolated from patient testicular biopsies. Within the testis, SSCs are present in a specialized niche on the basement membrane.

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in association with Sertoli cells. The SSC niche is responsible for providing key signals that are responsible for regulating SSC proliferation and self-renewal in vitro.

Hypothesis: We hypothesize that tissue-derived extracellular matrices (ECM) will provide critical niche factors and promote SSC proliferation and self-renewal in vitro.

Methods: Undifferentiated spermatogonia were enriched using ITGA6-based magnetic activated cell sorting followed by differential plating on type I collagen. These cells were then introduced into culture systems with laminin, human testis ECM (htECM), porcine testis ECM (ptECM), porcine small intestinal submucosa (SIS), porcine urinary bladder matrix (UBM) as substrate or on STO feeder cells. Undifferentiated spermatogonia were quantified on day 7 and day 14 using UTF1 immunocytochemistry. htECM was the only conditions that retained a significantly higher number of UTF1+ cells after 2 weeks in culture compared to STO feeder cell cultures. We next used the htECM culture condition to test and compare MEMalba-based mouse Serum Free Medium (mSFM), StemPro-34 with knockout serum replacement and Iscove modified Dulbecco Serum free medium. Recovery of UTF1+ cells was greater from cultures in StemPro-34 medium.

Conclusion: StemPro-34 on htECM constitutes a basal serum free, feeder free SSC culture condition for iterative testing of growth factors/ concentrations, passing times and conditions, cell density and other factors. The serum free, feeder free culture system is also likely to be more compatible with future clinical applications.

This work was supported by grants from the US-Israal Binalational Science Foundation and the Eunice Kennedy Shriver National institute of Child Health and Human Development (HD 092084)

21 (Poster) DEVELOPMENT OF SPERMATOGENESIS IN THREE-DIMENSION CULTURE FROM SPERMATOGONIAL CELLS OF BUSULFAN-TREATED IMMATURE MICE.

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(Presented By: Mahmoud Huleihel, PhD)

Introduction: Aggressive chemotherapy may lead to permanent male infertility. Prepubertal males do not generate sperm to be cryopreserved for future fertility preservation. However, their tests contain spermatogonial cells (SPGCs) that could be used for fertility preservation. In our previous studies, we showed development of sperm-like cells in three-dimension (3D) in vitro culture.

Objectives: To examine the effect of busulfan (BU) treatment of immature mice on their SPGCs, and the possible induction of survival of SPGCs to develop spermatogenesis in 3D in vitro culture.

Methods: Seven-day-old mice were injected intraperitoneal (i.p) with BU (45 mg/kg) sacrificed after 0.5-12 weeks in an interval of 1 week. Testes were weighted and their histology was evaluated compared to control (DMSO). SPGCs (SALL4 and VASA) were counted (immunohistochemical staining) in testicular tissue. Cells were enzymatically isolated from seminiferous tubules (STs) and cultured in methylcellulose as a 3D in vitro culture, in the presence of KSR and growth factors and homogenates from immature or adult mice.

Results: A significant decrease (p<0.001) in the testicular weight and in the number of SALL4 and VASA cells (p<0.001), and a significant increase in the number of severe damaged STs were detected 0.5-8 weeks after BU injection compared to control (p<0.001). Isolated tubular cells from immature mice (10 days after BU injection or control; a point in time with nadir effect of BU) showed development of colonies 4 weeks after initiation of culture in methylcellulose. We also detected development of meiotic and post meiotic cells in the cultures as examined by specific markers for cells of each stage. Homogenates from adult GFP mice, which were frozen/thawed and filtered in 0.22 filters to excluded the presence of sperm were added to culture of tubular cells from BU-treated immature mice and were found to induce development of sperm-like cells, negative for GFP staining, after 4 weeks of culture.

Conclusion: We demonstrate for the first time, the presence of biologically active SPGCs in testicular tissue of BU-treated immature mice, and their capacity to develop in vitro to different stages of spermatogenesis including the generation of sperm-like cells. This study may open new therapeutic strategies for fertility preservation of chemotherapy-treated patients, and mainly prepubertal males.

22 (Poster) A COMBINATION OF GINGER, PAULLINIA CUPANA, MIURA PUAMA AND L-CITRULLINE ACTIVATES THE INOS-SGCG-CGMP PATHWAY IN CAVERNOSAL SMOOTH MUSCLE CELL CULTURE

Monica G. Ferrini MS, PhD⁵, Andrea Abraham BS³, Sabine Nguyen DO³, Jorge N. Artaza MS, PhD⁴ and Jacob Rajfer MD²

¹Charles R. Drew University; ²David Geffen UCLA School of Medicine

(Presented By: Monica Ferrini, MS, PhD)

Introduction: It was recently reported that long-term daily treatment with a nutraceutical combination of ginger, Muira puama, Paullinia cupana and L-citrulline (COMB-4) enhanced erectile function in the aged rat with a significant improvement of the intracorporeal pressure following papaverine injection, a decrease in the drop rate as measured by cavernosometry and an increase in the cavernosal smooth muscle (CSM) content as well as the CSM to collagen ratio. In order to determine whether these pro-erectile and anti-fibrotic effects with COMB-4 could be the result of the modulation of the NO-cGMP pathway, an in vitro study using a rat corporal SMC culture was conducted.

Materials and Methods: Primary CSM cell cultures were initiated from the corpora cavernosa of 2 month-old rats. The CSM cells were grown in Dulbecco media with 20% fetal calf serum and then incubated with or without COMB-4 (ginger: 0.225 mg/ml; Muira puama, Paullinia cupana and L-citrulline each at 0.9 mg/ml) for 24 hours. mRNA and proteins were extracted and used for the determination of iNOS, eNOS, nNOS, and guanylate cyclase (GUICY1b2). cGMP content was determined by ELISA. Nitrites were measured by the Griess reaction. L-NIL (1mM) and L-NAME (3mM) were used as an inhibitor of iNOS and NOS activity, respectively.

Results: When COMB-4 treated CSM cells were compared to non-treated CSM cells, cGMP and nitrite levels were increased by 2 and 1.8 fold, respectively, after exposure to 24 hours of COMB-4. Both L-NIL and CSM to collagen ratio. In order to determine whether these pro-erectile and anti-fibrotic effects with COMB-4 could be the result of the modulation of the NO-cGMP pathway, an in vitro study using a rat corporal SMC culture was conducted.

Conclusion: These data demonstrate that the improvement in the morphological and physiological characteristics of the erectile mechanism by long-term treatment with COMB-4 in aged animals may be due to its stimulatory effect on the endogenous production of intracellular iNOS and GUICY1b2 resulting in the production of high levels of intracellular NO by the corporal CSM cells themselves. It remains to be determined whether these experimental observations as seen both in vivo and in vitro in the rat are translatable to any clinical outcomes regarding erectile function in adult men.
23 (Poster)
CIGARETTE SMOKE-INDUCED SPERM DNA METHYLATION (DNAm) CHANGES IN MICE AND PARTIAL RECOVERY FOLLOWING SMOKING CESSION
Kenneth Aston PhD¹, Patrick Murphy PhD², Timothy Jenkins PhD², Douglas Carrell PhD³ and Bradley Cairns PhD²
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(Presented By: Kenneth I. Aston, PhD)

Introduction: A growing body of evidence indicates that the sperm epigenome can be modified by environmental exposures, age and lifestyle factors. We recently reported significant changes to sperm DNAme in men who smoke compared with non-smokers. The reversibility of environmentally induced epigenetic changes has not been established.

Objective: To better characterize the dynamics and potential for correction of cigarette smoke-induced sperm DNAme changes, we exposed mice to cigarette smoke, and subsequently euthanized them for sperm collection and analysis, either shortly after completion of smoke exposure or after a recovery period of 7-8 weeks.

Methods: Treated mice were exposed to first-hand cigarette smoke for 7-8 weeks (n=18), and control mice were not exposed to smoke. Following the exposure period, some mice were euthanized shortly after removal from smoke (freshly smoked group; n=6), and the remaining animals were euthanized 7-8 weeks after removal from smoke (recovery group; n=12). Epididymal sperm were collecting by swim out, and sperm samples were processed for methylation analysis, including somatic cell lysis, DNA extraction and library preparation for reduced representation bisulfite sequencing (RRBS). Both CpG-wise and regional analyses were performed to identify differentially methylated CpGs (DMCs) and regions (DMRs) in smoke compared with non-smoked mice. DMCs were defined as cytosines that displayed >10% difference in methylation compared with controls. DMRs were regions comprising >3 CpGs with >5% absolute change in DNAme, while extreme DMRs (E-DMRs) displayed >20% change in DNAme. Comparisons between the number of DMCs, DMRs and E-DMRs in freshly smoked and recovery groups were made to determine whether smoke-induced DNA methylation changes diminish following a recovery period.

Results: In freshly smoked mice, we identified 96,165 DMCs and 9547 DMRs, while in the recovery group there were 76,431 DMCs and 6415 DMRs. More strikingly, 653 E-DMRs were identified in freshly smoked mice. DMCs, DMRs and E-DMRs in freshly smoked and recovery groups were analyzed to determine if they were associated with specific genomic regions.

Conclusion: These results indicate that cigarette smoke exposure significantly impacts sperm DNAme patterns. Reassuringly, the number of DMRs and the magnitude of methylation changes diminish significantly following a recovery period, indicating that environmentally-induced sperm DNAme may be corrected following the removal of the exposure.

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25 (Poster)
FUNCTIONAL COOPERATION BETWEEN THE TRANSCRIPTION FACTORS C/EBPß AND CJUN ON THE MOUSE INHIBIN BETA A SUBUNIT GENE IN LEYDIG CELLS
Nicholas Robert MSc¹, Gabriel Baron MSc² and Jacques J. Tremblay PhD²
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(Presented By: Nicholas Robert)

Fetal Leydig cells produce testosterone and insulin-like 3 that masculinize the embryo and promote testis descent. These cells also produce activin which is essential for testis cord morphogenesis. Leydig cell-produced activin A stimulates proliferation and inhibits differentiation of Sertoli cells. Activin A is also produced by adult Leydig cells and is believed to influence germ cell maturation during postnatal development. Activin A, a peptide belonging to the TGFβ protein family, is a dimer of inhibin-ßA, encoded by the Inhba gene. Nothing is known regarding the regulation of Inhba gene expression in Leydig cells. Our objective was to characterize the mechanisms of Inhba expression in these cells. A -1853 to +67 bp fragment of the mouse Inhba promoter was isolated and 5’ deletion constructs were generated and then transiently transfected in MA-10 Leydig cells. Deletion from -1853 to -144 bp had no impact on Inhba promoter activity, while further deletion to -65 bp caused a 70% reduction. Analysis of the -144/-65 bp region revealed the presence of several potential binding sites for transcription factors belonging to the CCAAT/enhancer binding protein (C/EBP) and CJUN families. We therefore tested whether C/EBPβ and CJUN could activate the Inhba promoter. Overexpression of C/EBPβ only weakly (1.5-2 fold) activated the -1853 bp Inhba promoter whereas in the presence of CJUN, a 7-fold activation was observed. Because of the close juxtaposition of the AP1 and C/EBP elements within the -144/-65bp region of the mouse and human Inhba promoter, we tested whether these transcription factors functionally cooperate on the Inhba promoter. Co-transfection of both C/EBPβ and CJUN led to a synergistic activation of about 15 fold. This cooperation was also observed between CJUN and all three C/EBP factors tested (α, β, δ), whereas only CJUN (not JUND or JUNB) could synergize...
with C/EBPβ. In MA-10 Leydig cells, siRNA-mediated knockdown of C/EBPβ and cJUN led to a 50% reduction in endogenous Inhba mRNA levels and in the activity a 1.853 bp Inhba reporter, further supporting the involvement of these two factors in Inhba expression in these cells. Finally, ChIP assays revealed that both C/EBPβ and cJUN are recruited to the proximal Inhba promoter region in MA-10 Leydig cells. Our current work provides novel insights into the regulation of Inhba expression, which encodes an important hormone for male sex differentiation and reproductive function. Supported by CIHR.

26 (Poster)
LUMINAL ATP AND ADENOSINE MODULATE V-ATPASE-DEPENDENT PROTON SECRETION IN EPIDIDYMAL CLEAR CELLS
Maria Agustina Battistone PhD, Maria Merkulova PhD, Maria Peraltu Bachelor, Nicolas DaSilva PhD, Dennis Brown PhD and Sylvie Breton PhD
Massachusetts General Hospital/Harvard Medical School
(Presented By: Maria Agustina Battistone, PhD)

A complex luminal intercellular communication network coordinates the establishment of an optimal acidic environment for sperm maturation in the epididymis. Clear cells (CCs) secrete H+ via the apical vacuolar H+-ATPase (V-ATPase). Principal cells (PCs) secrete ATP, which is then hydrolysed into adenosine by ectonucleotidases (ECToNs). Here we studied the roles of ATP and adenosine in the regulation of V-ATPase in CCs, and their modulation by luminal pH. We previously detected an increase in luminal adenosine and a reduction in ATP levels after perfusion of the cauda in vivo at 7.8 vs the control pH of 6.6, suggesting an increase in ATP hydrolysis at alkaline pH. Proteomic analysis of epithelial cell apical membranes (by LC/MS-MS) revealed the expression of several ECToNs: Ectonucleoside Triphosphate Diphosphohydrolase (ENTPD), Ecto-5'-Nucleotidase (NT5E) and Alkaline Phosphatase (ALP). RNA sequencing of cauda CCs, isolated by FACs (fluorescence-activated cell sorting) from B1-EGFP mice showed expression of ENTPD 1, 4, 5, 6 and 7, NT5E and ALPLPL2. Perfusion of the cauda with NT5E and ENTPD inhibitors (AMPCP and POM-1) at pH 7.8 decreased adenosine levels (33 and 47% vs Ctr, p<0.03), but the ALP inhibitor (tetrasirole) did not. Only the ENTPD inhibitor produced a 10-fold increased in ATP levels vs Ctr (p<0.05). Immunofluorescence (IF) and cell fractionation followed by Western blot showed that all inhibitors prevented the alkaline pH-induced V-ATPase apical membrane accumulation in CCs. Perfusion of the cauda at pH 7.8 stimulated V-ATPase-dependent H+ secretion, which restored the pH towards the control pH of 6.6. The EctoN inhibitors partially prevented this recovery (Ctr: 7.2±0.05, AMPCP: 7.5±0.03, POM-1: 7.6±0.04, tetrasirole: 7.6±0.01, p<0.01). Moreover, IF revealed that the ATP channel, pannexin1, is located in epididymal apical membrane, in agreement with our previous study showing inhibition of ATP secretion in PCs by its inhibitor CBX. Here, luminal perfusion at pH 7.8 with CBX prevented the luminal pH recovery (Ctr: 7.1±0.07, CBX: 7.6±0.03, p<0.05), and the alkaline pH-induced V-ATPase apical membrane accumulation. In addition, CBX decreased adenosine levels by 35% (p<0.05), suggesting the contribution of pannexin 1 in ATP secretion by PCs, followed by its hydrolysis to form adenosine. Our work provides novel insights into the regulation of Inhba expression, which encodes an important hormone for male sex differentiation and reproductive function. Supported by CIHR.

27 (Poster)
SPERM CAPACITATION IS ASSOCIATED WITH PHOSPHORYLATION OF THE TESTIS-SPECIFIC RADIAL SPOKE PROTEIN RSPH6
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(Presented By: Bidur Paudel)

Mammalian sperm needs to undergo a series of biochemical and physiological changes collectively known as capacitation as a prerequisite to fertilize. At the molecular level, capacitation induces a fast increase in cAMP and initiates a phosphorylation cascade. Although the increase in phosphorylation is well-established, the role of phosphorylation is not well understood. In the last years, tandem mass spectrometry has been used to identify exactly phosphorylated sequences and to compare their relative abundance in sperm incubated in conditions that support or not capacitation. In this work, a novel phosphopeptide corresponding to the radial spoke protein RSPH6 was found to be enriched in capacitated sperm. RSPH6 gene has six exons, five of which have been conserved in flagellated cells, including the algae Chlamydomona reinhardtii, during evolution. However, the exon containing the capase-dependent phosphorylation site was found exclusively in eutherian mammals. Transcription analyses revealed at least two different splicing variants; all of them are testis-specific. RSPH6 mRNA expression starts in spermatogonia; its mRNA continues to be present in spermatozoids. Using one of the RSPH6 N-terminal domain sequences, anti-peptide antibodies were generated. These antibodies localized RSPH6 in the sperm flagellum. Consistent with its role in the axoneme, solubility analyses revealed that RSPH6 is strongly attached to cytoskeletal structures. Studies in Chlamydomona reinhardtii predict that RSPH6 connect the central pair of microtubules with surrounding pairs. Altogether, the role of phosphorylation in the regulation of sperm motility, the finding that RSPH6 is phosphorylated during capacitation and its predicted asexual localization suggest RSPH6 as a candidate protein mediating signaling processes in the sperm flagellum.

28 (Poster) - WITHDRAWN

29 (Poster)
ROLE OF THE ARYL HYDROCARBON RECEPTOR IN THE DISTORTION OF THE SEX RATIO IN EMBRYOS Sired BY TCDD-ExPOSED MALE MICE
Kristin Bircsak PhD¹, Andrew Prantner PhD², Latresa Copes MS² and George Gerton PhD²
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(Presented By: George L. Gerton, PhD)

Introduction: Paternal exposure to the persistent organic pollutant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is associated with a skewed sex ratio towards a greater proportion of female offspring. Objective: This research aims to 1) determine AHR localization in the mouse testis and 2) following TCDD treatment, to identify the role of AHR in embryo sex ratio distortion. Methods: WT and Ahr knockout C57BL/6 male mice were injected weekly with TCDD (wk 1: 2000 ng/kg, wks 2-21: 400 ng/kg) or corn oil vehicle and mated with naïve female mice biweekly for 21 weeks. Embryos were collected at gestation day 15.5 and genotyped for sex. Male mice were sacrificed at 21 weeks and cauda epididymal sperm was collected for MP-FISH analysis of the percentage of X and Y chromosome-bearing sperm. Results: In the mouse testis, AHR was localized to the Leydig cells and post-meiotic germ cell populations, round spermatids, and elongating spermatids. Importantly, the sex ratio (number of males/total number of embryos) was significantly lower in embryos from WT TCDD-treated...
males relative to Ahr KO TCDD-treated males by 20% (Median values, WT Vehicle: 0.517; WT TCDD: 0.448; KO Vehicle: 0.550; KO TCDD: 0.557). These data suggest that paternal TCDD-mediated sex ratio distortion is mediated through AHR. Furthermore, 49.9-51.1% of the sperm carried the X-chromosome in all treatment groups verifying that there were not more X chromosome-bearing sperm in the WT TCDD-treated males.

**Conclusion:** Taken together, these data demonstrate a role for AHR in the female predominant embryo sex ratios observed in male mice exposed to TCDD and suggest that AHR in the testis may contribute to this phenomenon. In addition, the sex ratio distortion cannot be explained by an unbalanced production of X chromosome-bearing sperm by the TCDD-treated males. Supported by R21ES024527, P30ES013508, and T32ES019851.

30 (Poster)
**PATTERNS OF EPIGENETIC INSTABILITY PROVIDE NOVEL INSIGHT INTO HUMAN SPERM FUNCTION AS WELL AS POTENTIAL DIAGNOSTIC UTILITY**
Tim Jenkins PhD, Kenneth Aston PhD and Douglas Carrell PhD
University of Utah
(Presented By: Tim Jenkins, PhD)

**Introduction and Objectives:** DNA methylation is a key regulator of gene expression in many cell types. Human sperm have a unique epigenetic landscape with highly specialized methylation signatures. These methylation marks are strongly associated with patterns of expression in early spermatogenesis and appear to be altered in abnormal sperm.

**Methods:** DNA methylation was assessed in sperm samples from 96 normozoospermic donors. DNA was extracted and subjected to bisulfite conversion. The sperm DNA was then processed with Illumina 450k array and methylation patterns were recorded and analyzed. We performed a novel “epigenetic instability” analysis to assess regions of the genome with high levels of stability and those with low levels of stability between the samples in our study. In brief, we determined the absolute distance from the mean of each methylation mark in each individual. From this we were able to assess regions of the genome that were highly regulated/stable among different individuals as well as regions that were not highly regulated.

**Results:** Discrete patterns of epigenetic stability were identified in the group of donors that we assessed. Specifically, we found distinct regions that were most tightly regulated (displayed high stability) in the sperm from all men as well as regions that were poorly regulated (displayed a high level of instability). Based on pathway analysis, the most stable regions in sperm are associated with meiosis and spermatogenesis (this finding was highly significant with a -log10 pval = 67.95). In contrast, the regions that displayed the highest level of epigenetic instability between individuals were regions associated with immune function (-log10 pval = 19.4).

**Conclusion:** In this study, we found that patterns of stability in DNA methylation signatures between individuals were very significantly associated with cellular pathways important in sperm function. While not a surprising finding that methylation signatures reflect tight regulation of sites known to be important to spermatogenesis and cell function, this novel analysis technique (analysis of epigenetic stability) has very real potential diagnostic utility. This technique provides significant power to detect any movement away from a normal signature within discrete pathways and as such may be used as a screening tool in the future to predict abnormalities in spermatogenesis, fertilization capacity, and even embryogenesis.

31 (Poster)
**DIFFERENTIAL RNA EXPRESSION IN THE SPERM OF COUPLES WITH RECURRENT PREGNANCY LOSS**
Luke Simon PhD¹, Tim Jenkins PhD², Ki Aston PhD² and Douglas Carrell PhD⁴
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(Presented By: Luke Simon PhD)

**Introduction:** Recurrent pregnancy loss (RPL) is a condition where couples experience three or more consecutive pregnancy losses. Identification of a biomarker may prove beneficial for these couples, as in many cases the cause of RPL is unknown. Although, the functional relevance of RNA in sperm has been controversial, the recent development of RNA-seq and other molecular techniques, has enabled identification of a rich and complex repertoire of RNAs in mature sperm. The ability of these sperm borne RNAs to actively participate during early embryogenesis is not fully understood. However, some studies have demonstrated that selected sperm-derived RNAs contribute to early embryonic development.

**Objective:** The aim of this study is to identify differentially expressed transcripts in the sperm of patients with idiopathic RPL compared with fertile men.

**Methods:** Sperm of 16 men, including individuals with known fertility (n=8) and idiopathic RPL (n=8) with normozoospermic profile was used in this study. Total RNA was isolated using the QIAGEN RNA extraction kit according to the manufacturer’s recommendations following density gradient centrifugation and somatic cells lysis protocols. Small and long directional RNA-seq libraries were constructed according to Illumina’s protocol and RNAs were deep sequenced using an Illumina HiSeq 2000.

**Results:** Bioinformatic analysis revealed the presence of multiple classes of small RNAs in human spermatozoa. The total number of transcripts detected in our RNA-seq analysis in both the groups was 58,051, including mRNAs, IncRNAs, miRNAs, piRNAs, snoRNAs and snRNAs. Among the transcripts identified, thirty were underexpressed in the RPL group. Ontogenic analysis revealed that majority of the differentially expressed genes were protein coding genes associated with zinc finger proteins, regulatory proteins, receptors and nuclear complex proteins.

**Conclusion:** The results suggest a subtle but significant difference in the expression of transcript profiles between the fertile and RPL groups. These observations may be helpful to understand the presence of paternal RNA and serve as a molecular biomarker to identify patients with recurrent pregnancy loss.

32 (Poster)
**THE COMBINED EFFECT OF BMI AND AGING ON DNA METHYLATION SIGNATURES IN HUMAN SPERM**
Dallin Broberg BS, Tim Jenkins PhD, Kenneth Aston PhD and Douglas Carrell PhD
University of Utah
(Presented By: Dallin Broberg)

**Introduction and Objectives:** Significant alterations in sperm DNA methylation signatures are seen in men as they age. These patterns are strikingly consistent and as such are strongly predictive of age. In this study, we assessed the effects of BMI in general as well as at age-effected DNA methylation sites to determine whether or not patterns of age acceleration are present in an obese population.

**Methods:** Sperm samples from 96 men were used in this study. These samples were divided into 4 different age groups (22-24 years of age; 30 years of age; 40-41 years of age; and >48 years of age), which were each divided into two BMI categories (normal and obese) to enable us to detect the combined impact of BMI and age. DNA was extracted using Qiagen’s DNAeasy kit and subjected to bisulfite conversion via Zymo’s EZ DNA methylation kit. The sperm DNA was then processed with Illumina 850k (EPIC) array and methylation patterns were recorded and analyzed. We performed differential methylation analysis between the obese and
normal BMI groups in different age groups. Further, we assessed ‘germ line age’ with a recently constructed algorithm from our laboratory used to predict an individual’s age using sperm DNA methylation signatures. Results: DNA methylation data from each sample were used to predict an individual’s age; these predictions were used to determine if age acceleration patterns exist as a result of increasing BMI. Our results showed that individuals who were classified as obese based on BMI assessment (BMI > 30) were predicted to be approximately 4% older than their actual age when compared to individuals with a normal BMI (BMI = 19-25). This increase in predicted age was true for each age category but was highest in the youngest age category (~5% increased in predicted age).

Conclusion: In this study, we found that patterns of DNA methylation aging were more pronounced in patients with a high BMI compared to patients with a normal BMI within the same age category. As a result, predicted age was affected and found to be overestimated in high BMI samples. Future experiments analyzing each individual’s BMI history would be beneficial to further our understanding of BMI’s effect on DNA methylation signals and in particular to understand the nature of epigenetic aging in the gamete.

33 (Poster)
ENVIRONMENTAL PHENOL AND PARABEN EXPOSURE IS NEAR UBIQUITOUS AND AFFECTS SERUM TESTOSTERONE LEVELS IN HEALTHY ADULT MEN
Joseph Gabrielsen MD, PhD and Dolores Lamb PhD
Center for Reproductive Medicine, Baylor College of Medicine
(Presented By: Joseph Scott Gabrielsen, MD, PhD)

Introduction: Phenols and parabens are used in plastic food containers and cosmetic products, resulting in widespread exposure to the general population. Animal models and studies in pregnant women and children have found these compounds to be endocrine disruptors. Their effects on serum testosterone levels in adult men, however, are contradictory and poorly understood. We therefore sought to determine the association between serum testosterone levels and urinary levels of environmental phenols and parabens in healthy adult men.

Methods: Adult men from the 2011-2012 National Health and Nutrition Examination Survey who had serum testosterone and urinary phenols and parabens levels measured were included (n=840). Men were excluded if they had conditions/taking medications known to affect serum testosterone levels or did not self-report good health (n=170). Two men were excluded for testosterone below 75 ng/dL. Urinary phenol/parabens levels were normalized to urinary creatinine and log transformed to approximate normality. A linear regression model was created including BMI and age, with robust variance estimation due to heteroskedasticity. The model was then rerun to include a quadratic term to determine the best fit. Samples. Future experiments analyzing each individual’s BMI history would be beneficial to further our understanding of BMI’s effect on DNA methylation signals and in particular to understand the nature of epigenetic aging in the gamete.

Conclusion: Almost all men had detectable levels of urinary environmental phenols and parabens. The effects of these chemicals on testosterone were varied within each class; however, given near ubiquitous exposure to these chemicals, further research into their effects on the endocrine axis of adult men is critically needed. Source of Funding: JSG is supported by NIH K12 DK083014 Multidisciplinary K12 Urologic Research Career Development Program (to DJL).

34 (Poster)
TIMING OF SPERM CAPACITATION VARIES AMONG MEN AND IS CONSISTENT WITHIN MEN
G. Charles Ostermeier PhD, Cristina Cardona PhD, Melissa A. Moody MS¹, Alana J. Simpson BS¹, Romeo Mendoza BS¹ and Alexander J. Travis VMD, PhD
¹Androvia LifeSciences LLC; ²Cornell University College of Veterinary Medicine
(Presented By: Garry Charles Ostermeier, PhD)

Introduction: If inseminated too late, sperm may not complete capacitation before egg quality declines, preventing fertilization. Cap-Score® reflects the percent of sperm that can capacitate, as determined by GM1 distribution patterns. Here we compare differences in capacitation at two time points within semen samples, within and among individuals. We used ability to undergo acrosome exocytosis (AE) to confirm capacitation.

Methods: Semen from fertile men was liquefied, washed, and incubated for 3 hrs under capacitating (Cap) conditions, then fixed and analyzed immediately (Day0); after being incubated 3 hrs under Cap conditions then maintained 22-24 hrs in fix (Day1-fix); or after 22-24 hrs incubation under Cap conditions prior to fixation (Day1). In all cases, a light fixative previously shown to allow membrane lipid movements was used. A subset of the Day1 and Day0 samples (n=10) were treated with calcium ionophore A23187 and compared to samples incubated under Cap conditions without ionophore, to confirm capacitation. A set of the Day1 and Day0 samples (n=20) were treated with calcium and nystatin to confirm AE. Results: Phenols and parabens were detected in the urine of 96% and 98% of men, respectively. Urinary benzophenone-3, triclosan, and butyl parabens were inversely associated, while bisphenol A and methyl parabens levels were positively associated with serum testosterone levels after adjusting for age and BMI (Table 1). Ethyl and propyl parabens levels were not significantly associated testosterone levels in any of the models.

Conclusion: Almost all men had detectable levels of urinary environmental phenols and parabens. The effects of these chemicals on testosterone were varied within each class; however, given near ubiquitous exposure to these chemicals, further research into their effects on the endocrine axis of adult men is critically needed. Source of Funding: JSG is supported by NIH K12 DK083014 Multidisciplinary K12 Urologic Research Career Development Program (to DJL).
ABSTRACTS

35 (Poster)
ABNORMAL EXPRESSION, LOCALIZATION, AND TRAFFICKING OF MICRO-RNAS IN MEN WITH NONOBSTRUCTIVE AZOOSPERMIA
Russell Hayden MD, Anna Mielnik MS, Ryan Flannigan MD, Alexander Bolynakov MS and Darius Paduch MD, PhD
WCM
(Presented By: Russell Hayden, MD)

Introduction: The expression and localization of micro-RNA's are tightly controlled processes during normal spermatogenesis. We have previously reported marked differential expression of mir-34c and mir-202-5p comparing men with normal fertility and those demonstrating diffuse Sertoli-Cell Only Syndrome. In this study we expanded upon our pilot data by analyzing cellular localization of micro-RNA's in men with varying degrees of spermatogenic arrest.

Methods: Testicular biopsies were obtained from men undergoing testicular sperm extraction for infertility. Samples were snap frozen in liquid nitrogen for storage prior to processing. Specimens were independently read by two pathologists and classified as either: Sertoli-Cell Only (SCO), early maturation arrest (EMA), late maturation arrest (LMA), or normal (NL). In situ hybridization was conducted on representative slides that were deparaffinized and stained with probes synthesized by Exiqon; secondary antibodies linked to alkaline phosphatase were optimized with dilutions ranging from 400x to 800x. Scrambled probes were used as negative controls. A minimum of two runs were utilized per sample and images were processed with Lightroom (Adobe). Northern blots of promising micro-RNA's were conducted to confirm imaging results.

Results: Expression and localization were dependent upon histopathologic diagnosis. Mir-34c-5p demonstrated cytoplasmic staining within spermatagonia and spermatocytes in NL specimens. For mir-34c-5p, LMA was characterized by diminished staining and was absent in both SCO and EMA. Mir-202-5p and mir-202-3p were not expressed in SCO, and had markedly reduced expression for both EMA and LMA when compared against NL. Mir-202-5p was found predominately in the cytoplasm of Sertoli cells, whereas mir-202-3p was densely expressed in the nucleus of spermatocytes. Unlike NL specimens, mir-202-3p favored cytoplasmic staining over nuclear for samples with EMA and LMA. Northern blot confirmed that the detected mir-202-3p was compatible in size to mature micro-RNA. Mir-449c-5p was only expressed in NL specimens and mainly localized to the cytoplasm of spermatocytes.

Conclusion: Aberrant expression was observed for mir-34c-5p, mir-202-5p, mir-202-3p, and mir-449c-5p in men with deranged spermatogenesis. Nuclear staining of mature mir-202-3p suggests a noncanonical pathway. Abnormal biogenesis and trafficking of these micro-RNA's may prove key to the pathophysiology of spermatogenic arrest.

36 (Poster)
ANALYZING THE FUNCTION OF THE CAENORHABDITIS ELEGANS GENE M05D6.2, AN ORTHOLOG OF HUMAN T-COMPLEX PROTEIN 11 (TCP11), IN SPERM FUNCTION AND FERTILITY
Amber Jacob, Emily Lopes BS, Danielle Cooley and Matthew Marcello PhD Pace University
(Presented By: Amber Jacob)

Human t-complex protein 11 (TCP11) is a testis-specific gene product that is hypothesized to be necessary for proper sperm capacitation, acrosome reaction, and sperm morphology. M05D6.2 is the Caenorhabditis elegans ortholog of human TCP11. Our goal is to use the investigation of M05D6.2 gene function to understand the role of TCP11 in human reproduction. C. elegans have two sexes: hermaphrodite and male. Sperm from both hermaphrodites and males must undergo proper sperm activation, which includes processes similar to sperm capacitation and acrosome reaction in mammals, in order to migrate to and fertilize the egg. We have used RNA interference (RNAi) to disrupt the gene function of M05D6.2 in C. elegans. Hermaphrodites subject to M05D6.2 RNAi-treatment show no reduction in fertility. However, when male C. elegans are subject to M05D6.2 RNAi-treatment our preliminary results indicate that they have a significant decrease in fertility, despite making a normal number of sperm. We have generated three transgenic C. elegans strains using CRISPR/Cas9 genome editing (a deletion mutant, a mutant mimicking mutations found in infertile male patients, and a GFP-tagged version of the protein) to further characterize M05D6.2 function and localization. We are also investigating C. elegans strains with single nucleotide polymorphisms (SNPs) in the gene to characterize the function of specific residues in the TCP11 domain.

37 (Poster)
GENOME-WIDE STUDY OF NONOBSTRUCTIVE AZOOSPERMIA: NOVEL GENES AND NEW DIAGNOSTIC OUTLOOK
Alexander Yatsenko PhD¹, Nijole Pollock BS², Huaiyang Jiang MD³, Marta Olszewska PhD⁴, Tomas Jaffé MD⁴, Svetlana Yatsenko MD⁴, Joseph Sanfilippo MD³, Aleks Rajkovic MD, PhD⁴ and Maciej Kurpisz MD, PhD⁴
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(Presented By: Alexander Yatsenko, MD, PhD)

Infertility is a common reproductive disorder distressing ~15% of couples. Yet cause of male infertility and non-obstructive azoospermia (NOA) is mostly unknown; majority of men have a diagnosis of unknown (idiopathic) etiology. Current clinical genetic testing is informative in small fraction of infertile males. To improve the diagnostics, we performed extensive study of genomic aberrations and mutations in known coding genes. To perform the study, we combine two powerful tools, high-resolution array comparative genomic hybridization (aCGH) and massive whole exome sequencing (WES) and interrogate all RefSeq coding genes. We assemble 11 families and 289 sporadic cases diagnosed with NOA. To rule out known cause we tested NOA patients for chromosome aberrations and AZFs deletions. To test for invisible genomic aberrations, we utilize genomic 400K aCGH. To detect coding mutations we performed WES sequencing with SureSelectV6 capture library. We analyze genomic data using public and commercial software tools. Our initial study identified TEX11 gene mutations in 15% NOA patients with meiotic arrest. In following WES study of 78 NOA patients, we identified novel mutations in nearly 25%. These infertile patients include 12 males with X-linked hemizygous mutations and 9 with heterozygous mutations. Mutations were detected in TEX11, SYCP2, SYCE1, KISS1R, CIRBP, PRSS55, PTCCHD3, SERPINE2, TAF7L1, and GCNA1. These genes manifest in autosomal recessive and X-linked inheritance models. Interestingly, we found several NOA patients with potential polygenic inheritance; we noticed combinations of heterozygous mutations in several genes and aberrations in additional gene. To confirm pathogenic effect of the mutations, we began functional knock-out and knock-in studies in mouse. For future NOA diagnostic genomic testing of, we assembled initial gene panel. It includes genes with testis-specific expression in humans and animals, genes with critical meiotic and post-meiotic function. Our genomic studies discovered mutations in ~25% of NOA patients. Such high mutation detection rate is sufficient to warrant testing of any genetic illness. Thus, high detection rate and low cost of WES and CGH applications promise to become future clinical genetic testing for NOA. Our genomic results indicate wide spectrum of genes involved in NOA and its high genetic heterogeneity. We suggest that some mutations fit Mendelian single gene model, while other assemble in polygenic genetic model.
**ABSTRACTS**

**38 (Poster)**

**ANALYSIS OF SPERMATOGONIAL QUANTITY IN PEDIATRIC TESTES: ESTABLISHING REFERENCE VALUES AND A CLINICAL TOOL TO EVALUATE IMMATURE TESTES**

Stanley Kogan MD¹, Abinav Udayiar², Heather Barber², Demetri Hodges², Guillermo Galdon MD³, Nima Pourhabibi Zarandi MD², Kimberly Stogner-Underwood MD³, Shadi Quassem MD⁴, Anthony Atala MD⁵ and Hooman Sadri-Ardekani MD, PhD²

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(Presented By: Stanley J. Kogan, MD)

**Introduction:** Current rapid advances in reproductive medicine have generated new ability and interest in assessing fertility potential and preservation options in pre-adolescent boys at risk of infertility. Examples are boys with cancer where spermatogonia quantity can be affected negatively by radiation and chemotherapy; boys affected by environmental toxins, as well as those with developmental or genetic disorders. In adult patients, clinical pathology scoring systems such as Johnsen score or Kretser-Holstein score are used to evaluate levels of spermatogenic cells in the tests. Since spermatogenesis has not been started in immature testes, these scoring systems are not applicable to pre-pubertal testes.

**Objective:** Establish reference values and an automated clinical tool for evaluating age-related spermatogonia quantity in the testes of immature boys using a validated immunostaining system.

**Methods:** Our laboratory offers pediatric cancer patients an experimental option to bank testicular tissue to store spermatogonial stem cells (SSCs) for future clinical application to generate their fertility, thereby allowing opportunity to quantify spermatogonial cells in normal testes prior to exposure to gonadotoxic treatments. Patients having testicular tumors, testicular leukemic cell infiltration or gonadotoxic treatment prior to the biopsy were excluded from this study. Sections were stained with PGP9.5 (UCHL1) antibody, an undifferentiated spermatogonial marker, then scanned by NanoZoomer-XR Digital slide scanner. Using NDP.view2 Viewing software the number of basement and adluminal spermatogonia were counted.

**Results:** 48 testes were evaluated in patients ranging from 7 months to 12 years age and Tanner stages between I-IV. Average (±SEM) of basal spermatogonia cells/seminiferous tubule was 1.3 (±0.3) and 4.2 (±1.3) in boys <10 (pre-pubertal) and ≥10 years old (peri-pubertal). Average (±SEM) of adluminal spermatogonia/seminiferous tubule was 0.3 (±0.1) and 0.6 (±0.2) in boys <10 and ≥10 years old respectively.

**Conclusion:** We established an automated clinical pathology tool to quantify spermatogonial cells in immature testes based on immunostaining. To the best of our knowledge, this is the first clinical method establishing reference values of spermatogonial cell numbers by this method and that can be used in any diagnostic pathology lab to evaluate pediatric testis biopsies in pre-pubertal boys with fertility-related conditions.

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**39 (Poster)**

**DIRECT QUANTIFICATION OF FGFR2 MUTATION LEVELS IN SPERM OF CANCER PATIENTS AFTER CHEMOTHERAPY OR RADIOTHERAPY**

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(Presented By: Marvin L. Meistrich, PhD)

To study the mutagenic impact of chemotherapy and radiotherapy on male germ cells, we quantified mutation levels at positions c.752-755 in FGFR2 in sperm samples of 18 cancer patients that were obtained 1-22 years after therapy (and matched pre-treatment samples for 5 patients). When inherited, FGFR2 mutations at this location cause Apert (c.755C>G; p.S252W) or Crouzon (c.755C>T; p.S252L) syndromes and have previously been shown to be enriched (to levels ranging from ~10⁻⁶ to 10⁻³) in the sperm of men as they age. This is because these mutations confer a selective advantage to spermatogonial stem cells (SSCs) leading to their clonal expansion over time. All pretreatment samples showed mutation levels comparable to those observed in untreated individuals of similar ages. Surprisingly, the c.755C>G mutation levels in sperm from 7 patients treated with high doses of alkylating agents (6 cycles of MOPP, CVPP-ABDIC, CYADIC), which are potentially mutagenic and sterilizing, all showed extremely low mutation levels (<1x10⁻⁶) post-recovery. Of 4 patients treated with CHOP-Bleo (lower dose alkylating agents), 2 had age-appropriate mutation levels and 2 had mutation levels <1x10⁻⁶. Furthermore 3 patients receiving milder chemotherapy plus potentially sterilizing pelvic radiotherapy (1.9-4.2 Gy) also exhibited mutation levels <1x10⁻⁶ at their first post-treatment sperm analysis. The observed reduction in c.755C>G mutation levels suggest that Apert-mutant SSCs are more sensitive to high-dose chemo- or radiotherapy and are eliminated by such treatment. In contrast, of 4 patients treated with non-alkylating NOVP chemotherapy and/or radiotherapy (0.6 Gy to gonads) (i.e. considered to have a mild sterilizing effect on SSCs), 3 had c.755C>G mutation frequencies similar to controls, but one exhibited a rapid increase in mutation level (from 27x10⁻⁶ to 160x10⁻⁶ over one year), suggesting that resident Apert-mutant SSCs may have preferentially repopulated the depleted niches. Although we only assessed specific nucleotides (FGFR2 c.752-755), we show that cytotoxic therapy does not increase the FGFR2 mutation levels in sperm (in 17/18 patients) and may even reduce the mutational burden associated with age-related clonal expansion. These data are consistent with epidemiological studies that have demonstrated a low mutagenic risk for the progeny of male cancer survivors and should reassure patients contemplating reproduction several years after potentially mutagenic therapy.
ABSTRACTS

40 (Poster)
AMHR2-CRE-MEDIATED GLOBAL TSPO KNOCKOUT AND ITS ADVERSE EFFECT ON EMBRYONIC DEVELOPMENT, NEUTRAL LIPID ACCUMULATION AND REDUCED CIRCULATING TESTOSTERONE LEVELS IN THE ADULT
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(Presented By: Jinjiang Fan, MSci., PhD)

The rate-determining step in steroid biosynthesis is the transfer of cholesterol from intracellular stores into mitochondria where it is converted to pregnenolone by CYP11A1. This transfer is facilitated by the formation of a multiprotein complex composed of outer and inner mitochondrial membrane proteins, including the translocator protein (TSPO). TSPO is an ubiquitous high-affinity cholesterol binding protein, abundant in steroidogenic cells. Aberrant expression of TSPO is linked to various phenotypes, including neurological diseases, cancer and male reproductive aging. Recent studies of genetic deletion of Tspo in mice have provided conflicting data. Because of the clinical importance of TSPO, we re-assessed its role in a novel knockout (KO) mouse line. Amhr-Cre mice were previously used to generate Leydig cell-specific Tspo conditional KO (cKO) mice. However, using the same Cre mouse line, we reported an inability to generate Tspo cKO mice, possibly due to genetic linkage between Tspo and Amhr2 and the fact that Amhr2-Cre and Tspo are expressed at two-cell and morula stages of preimplantation development, respectively. We generated Amhr2-Cre-mediated Tspo global KO (gKO) mice by selective breeding scheme and genotyping, confirmed by immunofluorescence staining of various tissues. We also found that 33.3% of blastocysts at E3.5-4.5 showed normal morphology, whereas 66.7% of blastocysts showed delayed or abnormal development, which correlates with our expected proportions of Tspo+/+ (25%), Tspo-/- (25%), and Tspo+/ (50%) genotypes but only small number of mice (8.3%) with TSPO deficiency are normal. The Tspo gKO mice exhibited disturbances in neutral lipid homeostasis and reduced circulating testosterone levels. Re-analysis of RNA-sequencing data from various mouse tissues and analysis of new RNA-sequencing data revealed evidence of transcriptome changes in response to the loss of TSPO, including changes in several cholesterol-binding and transfer proteins. This study demonstrates that Amhr2-Cre can be used to produce Tspo gKO mice and can serve as a new global “Cre deleter” for any gene KO. Moreover, our results show that Tspo deletion causes abnormal and/or delayed pre-implantation embryonic development, alters adult neutral lipid storage, results in reduced androgen formation, and leads to transcriptome changes that may reflect compensatory mechanisms in response to the loss-of-function of TSPO. Supported by CIHR grants MOP125983 and PJT148659.
ROLE OF MITOCHONDRIAL TRANSLOCATOR PROTEIN (TSPO) MODIFICATIONS AND PROTEIN-PROTEIN INTERACTIONS IN MEDIATING THE EFFECT OF HORMONES AND THE TSPO DRUG LIGAND XBD173 TSPO ON LEYDIG CELL STEROID PRODUCTION

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(Presented By: Vassilios Papadopoulos, DPharm, PhD)

Steroidogenesis is a process through which cholesterol is converted to steroid hormones. The rate-limiting step in this process is the import of cholesterol molecules from intracellular stores to the inner mitochondrial membrane where CYP11A1 converts cholesterol to pregnenolone. This process is mediated by a protein complex composed of mitochondria-targeted cytosolic, and outer and inner mitochondrial membrane proteins that assist with the import and transfer of cholesterol molecules to CYP11A1. A component of this complex is the Translocator protein (TSPO), a ubiquitous high-affinity cholesterol binding protein that is abundant in steroidogenic cells. Abrupt expression of TSPO is linked to various phenotypes, including neurological diseases, cancer and male reproductive aging. TSPO binds with high affinity to diverse compounds that induce steroid formation in various tissues. Such compounds have been investigated as monitoring and therapeutic agents for various diseases. While the use of TSPO drug ligands is now in the clinic, the effect of the state of TSPO and its protein-protein interactions (PPIs) are not known. We characterized the TSPO microenvironment in the absence and presence of the high affinity TSPO drug ligand XBD173, a compound advanced to Phase III trials. Co-immunoprecipitation using phospho-serine and acetyl-lysine antibodies followed by immunoblot analysis using anti-TSPO antibodies indicated that following cAMP treatment, TSPO phosphorylation increases during steroidogenesis while protein levels and acetylation are not altered. We observed that in MA-10 mouse tumor Leydig cells, there are hormone-induced and time-dependent PPIs between TSPO and STAR and between TSPO and VDAC1. XBD173 induces steroidogenesis by altering these PPIs. To further test the drug specificity, we examined the effects of XBD173 on homozygotes (Tsopo/-) Sf-1-Cre driven Tsopo conditional KO and wild-type (WT) mice. Although XBD173 treatment increased steroidogenesis in WT mice, the effects seen was minor. However, treatment of XBD173 - exposed WT mice with hCG resulted in 2.5-fold higher induction of testosterone formation (10 – fold vs. 4-fold stimulation). This effect of XBD173 was not seen in Tspo KO mice. Taken together these data indicate that the state of TSPO and PPIs provide the specificity needed for drug ligands to activate TSPO-mediated steroid formation. Supported by grants from CHIR (MOP125983 and PJT148659) and NIH (2R01AG021092).
sterile and infertile. We conclude that the potential anti-cancer benefits that could result from the clinical use of GSK2879552 should be evaluated with caution concerning spermatogenesis and male fertility, and that the general focus of targeting histone demethylases and other epigenetic modulators to treat specific types of cancer should be tempered with the awareness that fertility might be impacted by such targeting strategies.

44 (Poster) PRIMARY CILIA: CELL ANTENNAS AND SIGNALLING HUBS IN THE EPIDIDYMIS.
Agathe Bernet, Olivia Jerczynski, Maira Bianchi Rodrigues Alves, Christian Roy, Laura Girardet, Alexandre Bastien, Claude Robert, Denis Soulet and Clemence Belleannee PhD U. Laval
(Presented By: Clemence Belleannee, PhD)

Introduction: Primary cilia (PC) are solitary microtubule-based antennas present at the cell surface. These non-motile cilia are ubiquitous and play an important role in organ development and tissue homeostasis through the transduction of signalling pathways. Although PC dysfunction is associated with Human male infertility issues (Hildebrandt, NEJM, 2011), the role of these organelles in the control of sperm fertilizing abilities remains unexplored.

Objective: The epididymis being in charge of post-testicular sperm maturation, our main goal is to define the role of PC in epididymis homeostasis and to evaluate their contribution to the control of sperm fertilizing abilities in this organ.

Methods: To this aim, we are using pharmacological and imaging approaches (confocal, STED) on organotypic cultures and transgenic mouse model presenting with endogenous fluorescence in PC (Arl13b-mCherry/Centrin2-GFP). In addition, we developed a conditional knock-out mouse in which Arl13b ciliary component is invalidated in primary cells from the epididymis (Krt5Cre-Arl13bfl/fl) to assess in vivo and wild type mouse in which Arl13b ciliary component is invalidated in primary cells from the epididymis (Krt5Cre-Arl13bfl/fl) to assess in vivo spermatogenic and epididymal functions.

Results: Our study demonstrates that PC are exclusively found associated with Keratin-V positive basal epithelial cells and alpha-actin positive peritubular cells in the epididymis of adult mice. Activation of the Hedgehog (Hh) signalling pathway with Indian (Ihh) and Sonic (Shh) agonists in the presence or absence of Ciliobrevin D, a blocker of the Hedgehog (Hh) signalling pathway with Indian (Ihh) and Sonic (Shh) agonists in the presence or absence of Ciliobrevin D, a blocker of the Hedgehog (Hh) signalling pathway in the epididymis. This pathway being known to regulate epididymid functions and sperm motility (Turner et al, J. Androl, 2006), in vivo studies of Krt5Cre-Arl13bfl/fl mouse strains and control littermates will further decipher the role of basal cell-PC in epididymal physiology.

Conclusion: Our research unravels for the first time the role of PC in epididymal signalling and their potential involvement in reproductive health. Our research could ultimately provide new avenues on the unexplored potential of ciliary components in the diagnosis and treatment of male infertility.

ABSTRACTS

45 (Poster) TITLE: DELETION OF KANK1 IN MALE MICE IS ASSOCIATED WITH DECREASED SPERMATOGENESIS
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(Presented By: Nannan Thirumavalavan, MD)

Introduction and Objectives: Copy number variant assays in patients with genitourinary birth defects revealed the gene encoding kidney ankyrin repeat-containing protein 1 (KANK1) as a candidate gene for these conditions, including ambiguous genitalia, micro-penis, ambiguous genitalia, cryptorchidism, and small testes to name a few. We aimed to further assess the phenotype of loss of function of the Kank1 gene in mice.

Methods: Kank1 homozygous null mice were created using a CRISPR/Cas9 system. All experiments were performed on both knockout mice and wild type mice as controls. Phenotyping was performed at 10 weeks of age. Mice were sacrificed at one year of age, then dissected to isolate their testes and epididymides. Testicular mass was measured, and histology assessed using hematoxylin and eosin staining. The cauda of epididymides were removed, minced and placed in 1cc of Human Tubular Fluid (HTF). Semen analyses were then performed, focusing on motility and sperm count to calculate the total motile count. All semen analyses were performed by a single investigator (NT), who was blinded to the Kank1 status of the mouse at time of analysis (wild type vs. knockout).

Results: Phenotyping the Kank1 null males at 10 weeks revealed micro-penis and an abnormally shaped mump in 1 knockout mouse, but no other abnormalities. No cryptorchidism was noted. A total of 8 null male mice and 6 control mice were sacrificed. Average testis mass (normalized to mouse mass) was significantly lower (.0015 % vs. 0.025% of body weight) for knockout mice (p=.029). Total motile sperm counts were also significantly lower (5.18 million/cc vs. 12.36 million/cc) in knockout mice compared to wild type mice (p=.039). Testis histology showed gross differences, including vacuoles in the testes (See image 1).

Conclusion: Evaluation of kank1 null mice has just begun and already demonstrates histologic differences and decreased spermatogenic function. Additional studies to assess fecundity with breeding studies, hormonal measurements, and morphologic assessment of sperm will be performed to define the role of Kank1 in male reproductive development and function.
46 (Poster)
HIGH-QUALITY SPERM RNA MOLECULAR BIOMARKERS TO ASSESS FERTILITY AND ENVIRONMENTAL TOXICANT EXPOSURES
Enrica Bianchi PhD, Angela Stermer PhD, Kim Boekelheide MD, PhD, Mark Sigman MD, Susan Hall and Kathleen Hwang MD
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(Presented By: Enrica Bianchi, PhD)

Gene expression profiling of mammalian sperm has been proposed as a novel non-invasive tool to evaluate male fertility and testicular toxicity. However, isolation of sperm RNA is a challenging procedure due to the unique biology of sperm and the heterogeneous population of cells present in the ejaculate, indicating the need for high quality control checks to ensure reproducibility of data generated from sperm RNA. Semen contains somatic cells, such as leucocytes and epithelial cells, along with spermatozoa that contain a very low abundance of RNAs compared to somatic cells. Therefore, somatic cell removal is essential to avoid contamination of sperm transcripts. The present study was designed to develop a reliable and effective protocol for RNA isolation from rat and human sperm that delivers highly purified and intact RNA, verified using RNA-specific electrophoresis and molecular biology approaches. To develop a standardized sperm RNA protocol, we evaluated quality and purity of rat and human sperm RNAs isolated using different sperm collection, purification and RNA extraction approaches. Our findings demonstrated that rat sperm RNAs isolated from epididymal fluid harvested using two collection approaches, microdissection and repeated needle puncture, showed 18S and 28S ribosomal peaks, and similarly showed that the somatic cell lysis buffer (SCLB) approach effectively removed all non-sperm RNAs compared to red blood cell lysis buffer (RBC). Proteins 2 (Prm2), a sperm specific marker, was significantly increased in SCLB-treated samples compared to RBC lysate buffer-treated samples. Differently, Cd45 and CdH1 transcripts and protein levels, selectively expressed in somatic cells, have not been detected in SCLB-treated samples. Complete lysis of rat sperm nuclei was obtained through lysis buffer with additional microbeads, and verified by microscope and enhanced sperm RNA yield. However, due to the interspecies differences in sperm morphology and chromatin condensation, human sperm completely dissolved after incubation with lysis buffer at room temperature without microbeads. The method we have optimized is suitable for comparative sperm transcriptomic analysis, such as sequencing and directed PCR. In conclusion, this RNA isolation protocol with across species adaptations improves reproducibility of functional genomics studies of pharmaceutical, chemical, and environmental exposures and infertility in pre-clinical and clinical setting.

47 (Poster)
VALIDATION OF CELLVISION DISPOSABLE 100 MICROMETER HEMOCYTOMETER WITH IMPROVED NEUBAUER RULING
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ANOVA, Karolinska University Hospital and Karolinska Institutet
(Presented By: Lars Björndahl, MD, PhD)

Introduction: Reliable sperm concentration assessments are crucial for the evaluation of male reproductive functions well as for the selection of best treatment modality of Assisted Reproductive Technologies. Recommendations by the WHO and the European Society of Human Reproduction and Embryology (ESHRE) are that representative aliquots of semen are diluted to immobilize spermatozoa and that sperm in the suspension are counted in a hemocytometer, for practical reasons preferably with improved Neubauer ruling. The non-disposable Neubauer chambers require considerable time for cleaning and cover slip application as well as routines to secure that wear and tear does not cause errors. We have earlier investigated disposable plastic chambers where the optical properties concealed significant numbers of spermatozoa.

Objective: To validate if disposable glass counting chambers with improved Neubauer ruling by CellVision give results that do not differ from routine analysis performed with conventional non-disposable chambers.

Methods: Method for sperm counting by WHO and ESHRE for both chambers was used – loading the same diluted sperm suspensions in both chamber types. In one series 25 consecutive samples were compared pair-wise. In another series the variability in conventional and CellVision chambers was examined by 10 replicate assessments of the same sperm suspension.

Results: Pair-wise comparison of counts in 25 consecutive samples (range: 8-295 millions/mL) showed an average difference between the two series of 4x10⁹/mL but the pair-wise difference was not significant (P=0.1688). For 10 replicate assessments of the same sperm suspension with the average concentration of 121.4 and 120.3x10⁹/mL, respectively, for conventional and CellVision, the variability for both chambers were satisfactorily low (range 115-127 and 106-130; coefficient of variation 3.41% and 5.44%).

Conclusion: The Cellvision chamber is as reliable as our routine non-disposable hemocytometers. The lower number of sperm (3%) found in the disposable chamber may be due to a small volume error in the conventional chambers, where chamber depth is not controlled for each application of a cover slip. CellVision chambers depth are guaranteed to be within ±3% by the manufacturer. Furthermore, we find the disposable chamber much easier to handle and use than the conventional. Also, the lack of cleaning and cover slip application speaks in favour of the disposable CellVision improved Neubauer counting chamber.

48 (Poster)
EPITHELIUM SPECIFIC TGFB1 TARGETING INDUCES LEUKOCYTOSIS IN MOUSE EPIDIDYMIS.
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(Presented By: Fernando Pierucci-Alves, DVM)

In the male body, immunogenic sperm are present long after central tolerance is established. This suggests peripheral tolerance mechanisms function within the male tract to prevent a sperm-targeted immune response and severe inflammation. These male tract tolerance mechanisms must be active especially in the epididymis, an organ that does not exhibit tolerance to allo- or xeno-grafts as the testis does. Importantly, the epididymis exhibits an abundant population of CD11c+ mononuclear phagocytes. Mice lacking TGFbeta-signaling in CD11c+ cells present severe epididymal leukocytosis and anti-sperm antibodies. The hypothesis driving this present study is that TGFbeta1, secreted by epididymal epithelia, physiologically suppresses the local immune response to sperm and maintains male tract health and reproductive function. To test this, we generated a Cre-Lox mouse (Tgfβ1Δepit) to delete Tgfβ1 or significantly reduce its expression in epididymal epithelia. To date, adult male Tgfβ1Δepit mice have been generated in mixed genetic background. Tgfβ1Δepit present reduced body weight compared to littermate controls, however no morbidity is apparent at 12 wks of age. Tgfβ1Δepit mice exhibit leukocyte infiltrations into the epididymal duct luminal space and cell suspensions derived from the entire Tgfβ1Δepit to littermate controls, however no morbidity is apparent at 12 wks of age. Tgfβ1Δepit mice exhibit leukocyte infiltrations into the epididymal duct luminal space and cell suspensions derived from the entire Tgfβ1Δepit epididymis reveal increased leukocyte (CD45+ ) numbers, as measured by flow cytometry. Although a more extensive phenotypic analysis is needed, these preliminary results are consistent with the experimental hypothesis. The kidneys of Tgfβ1Δepit mice also contain severe macroscopic pathology and very intense leukocytosis detectable by flow cytometry. The Cre line employed knowingly drives Cre expression in renal collecting duct epithelia. These data suggest the Tgfβ1Δepit mouse provides a non-technical strategy resource in our program, with potential to serve as a pre-clinical model of human pathologies such as leukocytospermia and antisperm antibodies. [Supported by P20GM103418 (K-INBRE); K-State Johnson Cancer Research Center; K-State Dept of Anatomy & Physiology; K-State College of Veterinary Medicine]
49 (Poster)

IN UTERO EXPOSURE TO BENZYLBUTYL PHthalate Disrupts Fetal Leydig Cell Development and Induces Multinucleated Gonocytes in Rats

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Introduction: Benzylbutyl (BBP) is one of phthalate plasticizers and used for many industrial products. Phthalates may cause Testicular Dysgenesis Syndrome (TDS), a condition caused by environmental endocrine disruptors to the fetal testis. TDS includes the cryptorchidism and hypospadias in the neonates and testicular cancer and infertility in the adult males. The objective of the present study was to investigate the effects of BBP on fetal Leydig cell distribution and function as well as gonocytes.

Methods: Female pregnant Sprague Dawley dams orally received vehicle (corn oil, control) or (10, 100, 500, and 1000 mg/kg/day) from gestational day (GD) 12 to GD 21. At GD 21, testicular testosterone production, fetal Leydig cell number and distribution, testicular gene and protein expression levels were examined. Testis multinucleated gonocytes were counted.

Results: BBP showed dose-dependent increase of the incidence of multinucleated gonocytes at 100 mg/kg. BBP dose-dependently increased abnormal fetal Leydig cell aggregation and decreased fetal Leydig cell size, cytoplasmic size at 10 mg/kg. BBP reduced the expression levels of steroidogenesis-related genes (including Lhcg, Star, Hsd3b1, and Hsd17b3) and testis-descent related gene Insl3 as well as protein levels of 3beta-hydroxysteroid dehydrogenase 1 (HSD3B1) and insulin-like 3 (INSL3) at 10 mg/kg. BBP significantly inhibited testicular testosterone levels at 100 mg/kg.

Conclusion: The results indicate that in utero exposure to BBP affects the expression levels of fetal Leydig cell steroidogenic genes and results in the occurrence of multinucleated gonocytes and Leydig cell aggregation. Key words: Benzylbutyl phthalate, fetal Leydig cell, Leydig cell aggregation, testosterone, multinucleated gonocytes. Funding: This work is supported by NSFC (81373032) and Zhejiang Provincial NSFC (LY15H310008).

50 (Poster)

AXONEMAL DYNEIN LIGHT INTERMEDIATE POLYPEPTIDE 1 FORMS A COMPLEX WITH PACRG IN THE MANCHETTE FOR CARGO TRANSPORT

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Introduction: Axonemal dynein light intermediate polypeptide 1(DNALI1) was originally cloned from Chlamydomonas reinhardtii in an effort to find motor proteins essential for flagellar motility. Earlier studies demonstrated that mouse DNAL11 protein is highly abundant in the testis. During the first wave of spermiogenesis, both Dnali11 mRNA and protein are dramatically increased at the spermiogenesis phase. Immunofluorescence studies demonstrated weak DNAL11 staining in late meioitic germ cells, but strong staining was found in round and elongated spermatids. DNAL11 has been identified as a binding partner of cytoplasmic dynein heavy chain 1 (CDHC1), which directly associates with microtubules. The protein is believed to be essential for motile cilia function. In our studies to investigate the mechanism of parkin co-regulated gene 1 (PACRG) in the regulation of spermiogenesis, we identified Dnali11 to be a PACRG binding partner. PACRG and meiosis expressed gene 1 (MEIG1) form a complex in the manchette, a transient and unique structure only present in the elongating spermatids for normal spermiogenesis. In transfected CHO cells, DNAL11 recruited the PACRG protein. PACRG alone is not stable when expressed in bacteria and transfected mammalian cells; however, co-expression of DNAL11 significantly increased PACRG expression level. When co-expressed in bacteria, non-tagged DNAL11 could be co-purified with His-tagged PACRG, and in the gel filtration assay, the two proteins were present in the same fractions. Immunofluorescence staining on the isolated male germ cells revealed that both DNAL11 and CDHC1 were present in the manchette of elongating spermatids, and DNAL11 and PACRG were co-localized in this structure. In the Pacrg knockout mice, localization of DNAL11 in the manchette was not changed. These observations strongly suggest that DNAL11 and PACRG form a complex in the manchette, with DNAL11 as an upstream molecular. DNAL11/CDHC1 may function as a motor system to drive MEIG1/PACRG complex to carry cargo proteins along the manchette microtubules for sperm flagella formation.

51 (Poster)

CHARACTERIZATION OF MOUSE INTRAFLAGELLAR TRANSPORTER PROTEIN IFT74 IN MALE GERM CELLS

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Introduction: Intraflagellar transport (IFT) is an evolutionarily conserved mechanism essential for the assembly and maintenance of most eukaryotic cilia and flagella. In mice, mutations in IFT proteins have been shown to cause several ciliopathies including male infertility. IFT74 is a component of IFT-B complex, and it forms a complex with IFT81, another IFT-B complex to transport tubulin for ciliogenesis. To study the role of IFT74 in sperm flagella formation and male fertility, we characterized its expression and localization in male germ cells. Mouse IFT74 is highly abundant in the testis and is also expressed in other somatic tissues enriched in cilia. During the first wave of spermatogenesis, the protein is expressed as early as day 8 after birth, and the expression level is dramatically increased during spermiogenesis. Immunofluorescence staining on isolated germ cells revealed that IFT74 is expressed as vesicles in spermatocytes and round spermatids. It is present in the manchette and centroosomes of elongating spermatids and the developing sperm flagella. These localizations suggest a role of IFT74 in sperm flagella formation. To investigate the role of IFT74 in male germ cells, the floxed Ift74 mice were bred to Stra8−/–Cre mice so that the Ift74 gene is disrupted in spermatocytes/spermatids. The Ift74: Stra8−/–Cre mutant mice did not show any gross abnormalities, and all of the mutant mice survived to adulthood. There was no difference between testis weight/body weight between controls and mutant mice. The effect of disruption of Ift74 gene in mouse germ cells on mouse spermatogenesis, sperm function and fertility is under investigation.

52 (Poster)

EFFECTS OF TRIPLOTIDE ON THE EXPRESSION OF ANDROGEN RECEPTOR IN HUMAN PROSTATE CELLS AND ITS MECHANISMS IN TRANSCRIPTION LEVEL

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Objective: The incidence of prostate cancer (PCa) showed a significantly rising trend in China. Triplotide (TP) is one of the major extracts from traditional Chinese medicine Tripterygium wilfordii, which can induce apoptotic effects on PCa cells with unclear mechanisms. Androgen receptor (AR) is a key target in the treatment of prostate cancer, and our previous study showed that TP can downregulate the expression of AR in PCa cells. This study is to investigate the mechanisms in downregulation of AR in PCa cells induced by TP in transcription level.

Methods: LNCaP cells were cultured and treated by TP in concentration of 0, 10, 30 and 50 nM, respectively. qRT-PCR and Western blot were used for many industrial products. Phthalates may cause Testicular Dysgenesis Syndrome (TDS), a condition caused by environmental endocrine disruptors to the fetal testis. TDS includes the cryptorchidism and hypospadias in the neonates and testicular cancer and infertility in the adult males. The objective of the present study was to investigate the effects of BBP on fetal Leydig cell distribution and function as well as gonocytes. BBP showed dose-dependent increase of the incidence of multinucleated gonocytes at 100 mg/kg. BBP reduced the expression levels of fetal Leydig cell steroidogenic genes and testis-descent related gene Insl3 as well as protein levels of 3beta-hydroxysteroid dehydrogenase 1 (HSD3B1) and insulin-like 3 (INSL3) at 10 mg/kg. BBP significantly inhibited testicular testosterone levels at 100 mg/kg.

Conclusion: The results indicate that in utero exposure to BBP affects the expression levels of fetal Leydig cell steroidogenic genes and results in the occurrence of multinucleated gonocytes and Leydig cell aggregation. Key words: Benzylbutyl phthalate, fetal Leydig cell, Leydig cell aggregation, testosterone, multinucleated gonocytes. Funding: This work is supported by NSFC (81373032) and Zhejiang Provincial NSFC (LY15H310008).
and AKT Thr308). The restriction-free cloning method was used to construct a series of pGL3-AR promoter reporter gene vectors which were transfected into LNCaP cells to investigate the effects of TP on the transcriptional activity of AR promoter. NF-κB inhibitor and Western blot were used to explore whether PI3K/ARK/NF-κB pathway is involved in the regulation of AR by TP. The data was analysed by GraphPad Prism 5.

Results: 1. The mRNA and protein expression of AR in LNCaP cells was dose dependent downregulated by TP. AR target gene PART1 and prostate specific antigen (PSA) mRNA was also downregulated.

2. A series of pGL3-AR promoter reporter vectors [pGL3-AR (-1767/+265bp), pGL3-AR (-1378/+265bp) and pGL3-AR (-965/+265bp)] were successfully constructed and validated by sequencing and luciferase activity.

3. The promoter reporter gene assay showed that TP could downregulate the expression of AR at the transcriptional level, and its regulation site located in -965/+265 bp of AR promoter.

4. PI3K/ARK/NF-κB pathway associated with AR promoter activity was dregregulated by TP.

Conclusion: Our results demonstrated that the transcriptional activity of AR in LNCaP cells was downregulated by TP, and PI3K/ARK/NF-κB pathway may be involved in the regulation mechanisms.

53 (Poster)

SYSTEMATIC IN-DEPTH PROTEOMIC ANALYSES REVEAL NOVEL PROTEINS ARE ENRICHED WITHIN HUMAN AND MOUSE TESTIS MITOCHONDRIA-ASSOCIATED MEMBRANES (MAM)

Shuiqiao Yuan PhD
Huazhong University of Science and Technology
(Presented By: Shuiqiao Yuan, PhD)

The past few years have provided novel insights into the existence of distinct contacts between the endoplasmic reticulum (ER) and mitochondria via contact sites known as the mitochondria-associated ER membrane (MAM). Increasing lines of evidence suggest that disrupted MAM structures or loss function of proteins at the MAM could lead to neurodegenerative diseases and cancer. However, the nature and characterization of the proteins from MAM in reproductive organ have not been identified yet. To better understand biological processes and molecular functions at the MAM in mammal testes. Here, we report a global mass spectrometry-based proteomic evaluation of the MAM obtained from human testes, mouse testes and brains. The evaluation and analysis showed that the components of MAM are highly conserved not only in different species (human testes and mouse testes) but also in different tissues (mouse testes and mouse brain), whereas MAM proteins in same tissues from different species (96.57%) are more conserved than that in different tissues form same species (60.82%). Bioinformatics interrogation of these protein catalogues using Ingenuity Pathway Analysis uncovered 815 new potential linkages are specific exist in same tissues from different species (96.57%) are more conserved than not only in different species (human testes and mouse testes) but also in different tissues (mouse testes and mouse brain), whereas MAM proteins in same tissues from different species (96.57%) are more conserved than that in different tissues form same species (60.82%). Bioinformatics interrogation of these protein catalogues using Ingenuity Pathway Analysis uncovered 815 new potential linkages are specific exist in mouse testis MAMs compared with mouse brain MAMs. Of note, a large portion of MAM proteins in testes have been reported to cause male infertility by gene knockout studies, such as DAZAP1, SPACA1. We also found that 1,350 proteins are highly conservative expressed at both human and mouse testes, whereas only 4 proteins are unique existed in mouse testes and 44 proteins are found expressed in human testes specifically. GO and KEGG analysis further revealed that top 10 biological pathway in mouse testis MAM specific proteins are related to spermatogenesis, male gamete generation as well as sexual reproduction. Based on our results, we postulate that the dysfunction of testis MAM proteins could affect spermatogenesis process by altering diverse cellular responses that further impaired male fecundity, and the proteins of MAM in testes may play essential roles in spermatogenesis and male fertility. Together, our data provide, for the first time, a variety of clues to define the relationship between testis MAM proteins, molecular pathways and reproductive diseases.

54 (Poster)

KCTD13 GENE DOSAGE CHANGES RESULT IN PENILE AND TESTICULAR ANOMALIES VIA AN ABERRANT ANDROGEN RECEPTOR SIGNALING.

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Baylor College of Medicine
(Presented By: Carolina Jorgez, PhD)

Objective: The molecular basis for hypospadias and lower genitourinary (GU) defects is poorly understood. Copy number variants (CNVs) in the potassium channel tetramerization domain containing 13, KCTD13 at 16p11.2 were identified in a subset of patients with hypospadias and cryptorchidism. KCTD13 is a substrate-specific adapter of the BCR-E3 ubiquitin-protein ligase complex. We tested the hypothesis that gene dosage changes in KCTD13 result in anomalous development of the lower GU tract via dysregulation of androgen receptor (AR) action.

Methods: Genomic DNA from pediatric patients with hypospadias and/or cryptorchidism, and boys without GU defects was analyzed by array competitive genomic hybridization (aCGH). Kctd13 null and haploinsufficent mice were generated using CRISPR genome editing and detailed phenotypic analyses were conducted.

Results: Two patients with hypospadias and cryptorchidism had CNVs covering KCTD13 identified using aCGH. We identified 27 other patients in the literature and public databases (DECIPHER) as well as 5 additional hypospadias and/or cryptorchidism patients in our cohort with KCTD13 CNVs. CNVs in KCTD13 were significantly more common in patients with GU anomalies compared to normal controls (p=0.008). Diminished KCTD13 expression results in significantly increased cytoplasmic AR levels and decreased nuclear AR levels in HeLa cells. KCTD13 knockdown in LNCaP cells decrease the mRNA levels of two AR-dependent genes (NRG1 and SGK1) suggesting that loss of KCTD13 downregulates AR downstream activity. Mouse phenotypic analysis revealed a significantly increased of cryptorchidism in Kctd13-/- and Kctd13+-/- mice compared to wild-type (WT) mice (p<0.05). The testis size and weight of Kctd13-/- and Kctd13+-/- displayed a significant decrease compared to WT mice. Fertility analysis of Kctd13-/- mice revealed subfertility with decreased sperm count and motility in comparison to WT mice (p<0.05). Penile MUMP and baculum length, and baculum base width were significantly smaller in null mice compared to WT mice (p<0.05).

Conclusion: KCTD13 regulates AR signaling axis in vitro. Mice with haploinsufficiency or homozygous deletion of Kctd13 exhibit penile and testicular anomalies, mimicking the phenotype seen in humans with CNVs involving KCTD13. The results suggest that Kctd13 impacts AR action and additional studies are required to define the molecular consequence of these CNVs.

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55 (Poster)

FLOW CYTOMETRY ANALYSIS REVEALED A RAPID INCREASE IN INTRACELLULAR CALCIUM IN A SUBPOPULATION OF MOUSE SPERM DURING CAPACITATION

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(Submitted By: Guillermima M. Luque)

Ejaculated mammalian sperm do not have the ability to fertilize oocytes. They must undergo a functionally defined process called capacitation. Sperm become capacitated in vivo by interacting with the female reproductive tract or in vitro in a defined capacitation media that contains bovine serum albumin (BSA), calcium (Ca2+) and bicarbonate (HCO3-). In this work, flow cytometry was used to analyze changes
in intracellular Ca2+ concentration ([Ca2+]i). For this purpose, sperm were double stained with propidium iodide (PI) and the Ca2+ dye Fluo-4 AM to analyze these changes in individual live sperm. An increase in [Ca2+]i was observed in a subpopulation of capacitated live sperm when compared with non-capacitated ones. Sperm exposed to capacitating medium displayed a very rapid increase in [Ca2+]i within 1 min of incubation, which remained sustained for 90 min. These rise in [Ca2+]i after 90 min of incubation in capacitating medium was evidenced by an increase in the normalized median fluorescence intensity (MFI). This increase was dependent on the presence of extracellular Ca2+ and at least in part reflected the contribution of a new subpopulation of sperm with higher [Ca2+]i. In addition, it was determined that the capacitation-associated [Ca2+]i increase at 90 min was dependent of CatSper channels, as sperm derived from CatSper knockout (CatSper KO) or incubated in the presence of CatSper inhibitors failed to increase [Ca2+]i. Altogether, these results indicate that a subpopulation of sperm increases [Ca2+]i very rapidly during capacitation due to a CatSper-mediated influx of extracellular Ca2+.

56 (Poster)
ROLE OF SERINE PROTEASES (PRSS50) IN SPERMATOGENESIS
Minerva Solis, Joshua Moore MS, Juan Bournat PhD, Adam Szafrań MD PhD, Jason Scovell BS and Carolina Jorgez PhD

Introduction and Objective: The role of meiotic testis-specific proteins is not well known. In silico, we identified PRSS50 a serine protease as a candidate required for sperm meiosis.

Methods and Results: We identified PRSS50 as a spermocyte specific protein expressed from post-natal day 14 (beginning of meiosis) to adulthood; it is also expressed in the sperm midpiece. To study the in vivo role of PRSS50, a Prrs50 knock-out (KO) mouse was generated using CRISPR/Cas9. Prrs50-KO male mice were severely sub-fertile; they also show a phenotype with variable penetrance. Testicular histology range from normal to Sertoli cells only within a single testis. Prrs50-KO testis have an abnormal number of residual bodies, germ cell distribution, and multi-nucleated spermatids. Motility was severely affected in the Prrs50-KO sperm due to a significant number of tail defects, ranging from two-headed sperm to sperm with multiple tails. We used high content analysis (HCA) to quantify defects in active sperm midpiece mitochondria using JC-1 labeling, a green-red dye sensitive to mitochondrial membrane potential. HCA combines the use of automated microscopy and image analysis algorithms to image, segment, and measure thousands of individual sperm per sample in an un-biased manner. Sperm from Prrs50-KO mice had a smaller total active mitochondrial area per sperm (806±12 vs 1146±27 pixels, p<0.01). Two predominant mitochondrial morphology patterns were observed and quantified using a cross-validated random forest artificial intelligence model. Sperm from Prrs50-KO mice had a significantly higher frequency of the truncated “abnormal” phenotype when compared to WT mice (65.3±0.3 vs 10.2±0.4, p<0.001). No difference in chromatin structure or DNA fragmentation could be detected between sperm populations using an HCA based sperm chromatin structure analysis (SCSA) in sperm labeled with acridine orange. To evaluate microstructural defects testicular electron microscopy (EM) was performed. Testis from Prrs50-KO mice have sperm with multiple axonemes in one cell membrane, and multi-nucleated cells in which the acrosome is already formed but the membrane dividing the cells has not formed.

Conclusion: Prrs50-KO mice are a unique sub-fertile model with abnormal testicular and sperm morphology. We are in the process of determining the role of Prrs50 in controlling either cytokinesis or spermiogenesis which will allow us to understand how proteases regulate meiosis and sperm formation.

57 (Poster)
SUBCLINICAL GENITOURINARY INFECTION SUSPECTED BY PHYSICAL EXAMINATION INCREASES REACTIVE OXYGEN SPECIES IN SEMINAL PLASMA: AN INITIAL REPORT
Jorge Hallak MD, PhD¹, ², ³, ⁴, ⁵, Rosa Monteiro BSc¹, ², ³ and Juliana Pariz PhD², ³, ⁴, ⁵

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Introduction: Genitourinary tract infections are the most common disease affecting male reproductive health, frequently do not have clear symptoms, therefore are not properly investigated neither diagnosed nor treated. Seminal leukocytes in response to subclinical infections can be the source of reactive oxygen species (ROS) that affects semen quality.

Objective: To study the influence of subclinical genitourinary in semen parameters and reactive oxygen species levels.

Methods: Twenty-seven semen samples of patients evaluated between 2012 and 2016 who not presented with any alteration on anamnesis and/or physical examination: pain in the external genitalia, symptoms of urethritis, burning sensation in the perineum, urethral discharge, pain, etc. After initial evaluation, a prostatic massage followed by microbiological analysis on urethral secretion (collected by swab), urine (medium-jet urine) and semen (collected by masturbation). Seminal parameters were evaluated by World Health Organization criteria (2010) and ROS level by chemiluminescence method.

Results: The bacteria frequency was Enterococcus spp. 48.1% (n=13), Staphylococcus aureus 22.2% (n=6), Klebsiella spp. 11.1% (n=3), Escherichia coli 14.8% (n=4), Mycoplasma spp. 7.4% (n=2), Ureaplasma spp. 7.4% (n=2), Gardnerella spp. 3.7% (n=1), Staphylococcus saprophyticus 3.7% (n=1). We did not observed important alterations in semen volume (2.87±2.22ml), pH (8.11±0.29), total sperm number (146.55±143.93 million), total progressive sperm number (49.79±65.41 million), total motility (51.85±21.22%), sperm concentration (60.93±58.00 million/ml) and normal morphology by WHO criteria (7.59±4.70%). The mean of progressive motility (30.18±21.05%) characterized asthenozoospermia. Was observed low normal morphology by Kruger criteria (1.26±1.63%). Patients presented round cells (2.69±4.97 million) and semen leukocytes (1.10±2.73 million). Was observed significant increase in seminal ROS level (6.70±15.80 x 104 cpm/20x106 sperm, range of 0.55 x 104 cpm/20x106 sperm).

Conclusion: Subclinical male genitourinary tract infections should be a concern by the andrologists when seeking for a diagnosis and correct treatment for male infertility, because even without symptoms, these infections affect negatively the seminal parameters essential for successful pregnancy, such as sperm motility and morphology, possibly by excessive ROS generated by leukocytes.

58 (Poster)
SNP AND ADAR MODIFICATION ANALYSIS OF MICRO-RNA’S IN 54 MEN WITH MALE-FACTOR INFERTILITY
Russell Hayden MD, Ryan Flannigan MD, Alexander Bolyakov MS, Anna Mielnik MS and Darius Paduch MD, PhD

WCM

Introduction and Objective: The regulation and function of micro-RNA’s are dependent upon a highly conserved local sequence. We have previously reported aberrant expression of micro-RNA’s when comparing men with Sertoli-Cell Only Syndrome against men with normal fertility. In this study, we evaluated for single nucleotide polymorphisms (SNPs) and other internal modifications that may inhibit micro-RNA function.

Methods: Testicular biopsies were obtained from men undergoing testicular sperm extraction for infertility. Samples were snap frozen in liquid nitrogen for storage prior to processing. Specimens were
ABSTRACTS

independently read by two pathologists and classified as either: Sertoli-Cell Only (SCO), early maturation arrest (EMA), late maturation arrest (LMA), or normal (NL). Total RNA was isolated and checked for purity and integrity. Non-stranded RNA-seq libraries were prepared (TRUsseq, Illumina) and sequenced on an Illumina HiSeq 2000 platform. Sequencing data was processed with Chimira (v1.5, EnrightLab).

**Results:** A total of 54 biopsies were procured from men with infertility with histopathologic diagnoses consisting of: 7 Klinefelter syndrome, 11 Sertoli-cell only syndrome (SCO), 11 early maturation arrest (EMA), 5 late maturation arrest (LMA), 10 hypospermatogenesis (HY), and 10 normal (NL). Chimira analysis demonstrated a variety of SNP and ADAR modifications among the sequences housed in miRbase (release 21). When comparing SCO against NL samples, we observed a highly prevalent U-SPAN at the 3’ end when globally summing all micro-RNA modifications across all examined micro-RNA sequences.

**Conclusion:** We observed a heterogeneous group of SNP and ADAR modifications when examining micro-RNA’s from our cohort of 54 men with male-factor infertility. A prevalent U-SPAN near the 3’ end was observed in men with SCO when globally assessing micro-RNA sequences derived from miRbase. The significance of these modifications warrants further investigation, as they may prove fundamental to the pathogenesis of disrupted spermatogenesis.

59 (Poster)
A NEW SPERM EF-HAND PROTEIN, EFCAB9, IS ASSOCIATED WITH CATSPER CHANNEL AND ESSENTIAL FOR MALE FERTILITY
Jae Yeon Hwang PhD and Jean-Ju Chung PhD
Yale School of Medicine
(Presented By: Jean-Ju Chung, PhD)

Sperm-specific Ca2+ channel, CatSper, is comprised of minimum 9 subunits, mediates hyperactivated motility in mammalian sperm. Our screen identified an uncharacterized testsis-specific protein with two EF-hand Ca2+ binding domains, Efcab9, as a candidate Ca2+ signaling molecule downstream of CatSper channel. Here we report that Efcab9 interacts directly with one of CatSper accessory subunits, CatSperζ, and expresses in a CatSper dependent manner in sperm cells. Efcab9-deficient males are subfertile due to defective flagellar bending and motility.

60 (Poster)
PHTHALATES AFFECT HUMAN TESTIS RETINALDEHYDE DEHYDROGENASE 1 MEMBER A1 AS THE POTENTIAL ENDOCRINE DISRUPTORS
Erpo Tian MD,1 Fei Ge MD,1 Renshan Ge MD2 and Ying Zhong MD3
1Jinjiang Maternity and Child Health Hospital; 2The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Erpo Tian, MD)

**Introduction:** Many environmental endocrine disruptors could increase incidence of both male and female infertility. The exposure to these chemicals may inhibit some critical enzymes for germ cell development. One family of these enzymes is retinaldehyde dehydrogenase (ALDH). Retinaldehyde dehydrogenases, especially ALDH1, ALDH2 and ALDH3, are responsible for catalysis of the formation of retinoic acid. Retinoic acid is critical for the development of germ cells. One member ALDH1A1 is critical for the earlier stage development of germ cells.

**Methods:** In the present study, we investigated different phthalates for their inhibition of human ALDH1A1 and the inhibitory mode of action and compared their structure-a-response. We cloned human ALDH1A1 and used their inhibition of human ALDH1A1 and the inhibitory mode of action and measured the formation of NADH to judge the effects of phthalates.

**Results:** At 100 μM, some phthalates potently inhibited ALDH1A1, with di-2-ethylhexyl phthalate (DEHP), Di-n-octyl phthalate (DNOP) and diisononyl phthalate (DINP) as the potent inhibitors, which inhibited the enzyme over 50%. Other phthalates almost did not inhibit the enzyme at 100 μM. DEHP, DNOP, DINP had IC50 value of 24,873, 51,915, 2,269 uM. DEHP, DNOP, DINP competitively inhibited human ALDH1A1 when propionaldehyde was used as the substrate and inhibited the enzyme in a mixed mode when cofactor NAD+ was used. Molecular docking study demonstrated that DEHP, DNOP and DINP bound to the propionaldehyde binding pocket of the enzyme with the low energy.

**Conclusion:** Our data clearly shows that only phthalates with 8-9 carbon numbers at alcohol moiety have the inhibitory action of human ALDH1A1. Other phthalates do not have inhibitory effects on ALDH1A1.

**Funding:** This work is supported by Health & Family Planning Commission of Zhejiang Province (11-CX29). Corresponding author Ying Zhong: yzhong08@yahoo.com and Renshan Ge: r_ge@yahoo.com.

**Keywords:** retinoic acid; human ALDH1A1; phthalate, competitive inhibition

61 (Poster)
ADJUDIN-LOADED NANOCAPSULES IN CONTRACEPTION OF MALE RATS
Chao Li Phd, Baiping Mao PhD and Renshan Ge Professor
(Presented By: Chao Li, Sr.)

**Introduction:** Previous studies have proven that the Adjudin is the potent male contraceptive, but with close discrepancy between 50% effective dose (ED50) and 50% lethal dose (LD50) (LD50/ED50, therapeutic index, TI). It is necessary to develop the new formulation of Adjudin with higher safety and increased TI.

**Objective:** To prepare the Phorbol 12-myristate 13-acetate (PMA) based Adjudin loaded nanocapsule, and evaluate the contraceptive effect and the TI in male rats.

**Methods:** The PMA-based Adjudin loaded nanocapsules (PMA-Adjudin) were prepared by oil-in-water method. The size distribution, loading efficiency, release properties of PMA-Adjudin were evaluated in vitro, the effectiveness and toxicities were determined in Sertoli cells. The regulation of PMA-Adjudin to Pim1 and Wnt4, and the effects of androgen production were systematically assessed in male rats in vivo.

**Result:** The hydrodynamic size of PMA-Adjudin and Adjudin loading efficiency were measured 425 ± 82 nm and 67% respectively. 0.8 ng/ml of PMA-Adjudin (Adjudin: 0.2 ng/ml) could effectively induce the apoptosis of the Sertoli cells. ED50 and LD50 of PMA-Adjudin to Sertoli cells were 0.2 ng/ml and 50 ng/ml (Adjudin) respectively. Compared with traditional Adjudin administration (dissolved in DMSO), the TI was increased by 21 times. The in vivo experiments demonstrated that single administration of 100 ng/kg of PMA-Adjudin (Adjudin: 25 ng/kg) can effectively induced serum testosterone decline on the 3rd day, reached the lowest level on the 7th day, maintained the low level 21 days and gradually increased to normal level at 28 days. The WB and RT-PCR manifested the increased gene and protein expression of Wnt4 and decreased levels of Pim1.

**Conclusion:** the PMA-Adjudin nanocapsule can effectively inhibit the formation and maturation of spermatozon by regulating the expression of Pim1 and Wnt4 in vivo, and increased the TI of Adjudin at least 21 times. The PMA-Adjudin is an ideal formulation for male contraception with lower toxicity and better safety.

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62 (Poster)
SPERM DNA DAMAGE: CONSEQUENCES OF THE IMPACT OF YOGIC COGNITIVE BEHAVIOR PRACTICES
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Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Shilpa Bish, MSc)

**Introduction:** Primary infertile men are predisposed to develop psychological distress such as major depressive disorder (MDD) and are also at the risk for poor quality of life (QOL) and poor sperm quality (sperm DNA damage) due to cellular aging.
ABSTRACTS

Methods: Primary infertile men (N=24) and healthy controls (N=20) were randomly assigned to a 21-week yoga intervention. Blood and semen sample was obtained from all the participants pre-and post-yoga intervention. Assessment of QOL and depression severity was done by WHOQOL-BREF scale and Beck Depression Inventory—II (BDI-II) scale respectively. Basic semen analysis based on WHO (2010) guidelines was also performed. Evaluation of improvement of sperm quality (DNA fragmentation index (DFI), reactive oxygen species (ROS) and total anti-oxidant capacity (TAC)) was done as the primary outcome. Secondary outcomes included improvement in QOL (WHOQOL-BREF scale) and reduction in depression severity (BDI-II), cellular aging and neurodegeneration (cortisol, ROS, TAC, telomere length, and brain derived neurotrophic factor (BDNF)).

Results: In linear mixed models, the yoga intervention led to significant improvements in basic semen analysis parameters based on WHO (2010) guidelines and significant reductions in sperm DFI (p<.003). Compared to non-yoga group, the ROS levels get lowered in the yoga group (p = 0.006), whereas the TAC levels were higher in the yoga group (p = 0.001). The yoga intervention led to a significant improvement in biomarkers of cellular aging in the primary infertile men and in healthy controls. Yoga group showed significant reductions in depressive symptoms (p=0.031) and improvement in QOL (p=0.039), as well as significant reductions in cortisol and increase in other circulating biomarkers, which include, telomere length, TAC and BDNF at post yoga intervention (p<.05 for all).

Conclusion: A brief, yoga intervention demonstrated preliminary short-term efficacy in improving the QOL and biomarkers of cellular aging in the primary infertile men. Simple lifestyle interventions such as yoga and meditation can be used as an adjunct to modern medicine and may aid in improving overall health, reducing psychological distress and improving the sperm quality. Further research is needed to analyze effects of yoga on male infertility patients with MDD for benefits in fertility. Further research is still required to validate the results of the present study.

Financial Funding: ICMR, New Delhi, India.

63 (Posters) GRANULOCYTE COLONY-STIMULATING FACTOR REGULATES DIFFERENTIATION OF STEM LEYDIG CELLS Linchao Li MD, Yiyan Wang MD, Qingquan Lian PhD and Ren-Shan Ge PhD
The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University
(Presented By: Linchao Li)

Introduction: Granulocyte colony-stimulating factor (GCSF) is secreted by many immune cells and may be involved in the regulation of reproductive system. However, effect of GCSF on stem Leydig cell (SLC) development remains unclear. The objective of this study is to investigate the effect of GCSF on Leydig cell regeneration from SLCs in ethane-dimethane sulfonate (EDS)-treated rat model.

Methods: Adult male Sprague-Dawley rats were intraperitoneally injected 75 mg/kg EDS to eliminate Leydig cells and then rats were divided into three groups, in which they were intratesticularly injected GCSF (0, 10, 100 ng/testis/day) for 7 days starting on post-EDS day 14, followed by a single intraperitoneal injection of ethane dimethane sulfonate (EDS) to eliminate LCs, and LC regeneration process was monitored. And an in vitro seminiferous tubule culture system was introduced to investigate stem LC development.

Results: After a brief exposure to PFOS, the regeneration of LCs were impaired, as it showed that the lower serum testosterone (e.g. control value on post-EDS day 56 being 3.46 ± 0.39 ng/ml and those after 5 and 10 mg/kg PFOS treatment being 1.58 ± 0.47 and 1.15 ± 0.14 ng/ml, respectively), the reduced number of regenerated LCs, and the decreased expression levels of LC specific genes (Lhcgr, Scarb1, Star, Cyp1a1, Hsd3b1, and Cyp17a1) and their proteins. We also found that PFOS inhibited stem LC proliferation (lowering EdU incorporation rate) and differentiation (lowering testosterone production and down-regulating LC specific gene and protein expressions). After 7 days of in vivo PFOS treatment, Dessert Hedgehog was down-regulated and in vitro replacement of this factor can prevent the negative effects of PFOS on stem LC development.

Conclusion: A brief exposure to PFOS causes the direct or indirect inhibitions on stem LC development.

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65 (Poster) - WITHDRAWN

66 (Poster) HYPERCHOLESTEROLEMIA INDUCED SPERM DAMAGE IN RAT AND THERAPEUTIC POTENTIAL OF MUCUNA PRURIENS (LINN.)
Seppan Prakash PhD, Murgesh Anuradha PhD, Muhammed Ibrahim PhD, Mohanraj Karthik Ganesh PhD, Lakshmanan Ganesh PhD, Premavathy Dinesh MSc, Muthu Sakhthi Jothi MSc and Wangpam Shimray Khayimm MSc University of Madras
(Presented By: Prakash Seppan, MSc, PhD)

Introduction: Hypercholesterolemia represents a high risk factor for frequent diseases and it has also been associated with poor semen quality that may lead to male infertility. The present study was intended to analysis the nature of reproductive damage inflicted during intake of fat diet and evaluate the therapeutic effect of the Mucuna pruriens (M.pruiriens), a leguminous plant identified for its properties like aphrodisiac and improving fertility, including androgenic & anticholesterolomic properties.
Objective: To analyze therapeutic potential of the M. pruriens on altered sperm functional parameters and membrane and chromatin integrity damage in high fat diet-induced hypercholesterolemia in rats.

Methods: Study comprised of four groups: i.e. control, hypercholesterolemia induced rat (fed with high fat diet), hypercholesterolemia +M.pruilens and Control+M.pruilens (ethanolic extract of the seed at dose of 200 mg/kg b.w for 60 days). At the end of the experimental period sperm collected were analyzed for: motility, morphology and morphometry, chromatin integrity, estimation of enzymic & non-enzymic antioxidants, ROS level, LPO, DNA integrity by fluorescent staining and mitochondrial membrane potential/integrity.

Results: Hypercholesterolemia impair sperm motility and showed increased abnormal sperm (microcephalic, amorphous and acesphalic). Increase in sperm with cytoplasmic droplets, poor chromatin integrity and mitochondrial membrane permeability were key observations. High ROS levels noticed and indicative of compromised antioxidants defense system. This would lead to mitochondrial membrane leak and poor respiratory chain through deteriorated ATP production. The inability of the sperm cell or the epididymal system to overcome the excessive ROS insult produced by these abnormal sperm, thence leading to increase in the levels of LPO, DNA/chromosomal integrity damage in the hypercholesterolemic rats. Thus under hypercholesteroleic condition free radical mediated damage along with poor anti-oxidant defense system would substantially damage the sperm function and structure. These alterations were considerably reduced or recovered in hypercholesterolemic rats treated with M.pruilens. No adverse effects seen in control +M.pruilens.

Conclusion: These observations indicate the therapeutic efficacy of the M.pruilens seed extract towards controlling the multifaceted pathophysiological affecting sperm under hypercholesterolemia.

Funding: SERB, Govt. of India

68 (Poster) - WITHDRAWN

69 (Poster)
GUO’S SINGLE-ARMED SUTURE TECHNIQUE FOR MICROSCOPICAL VASOEPIDIDYMOSTOMY
Yiming Yuan MD & PhD, Hongen Lei MD, Zhichao Zhang MD, Jing Peng MD, Wanshou Cui MD and Zhongcheng Xin MD
Andrology Center & Urology Department, Peking University First Hospital
(Presented By: Yiming Yuan, MD, PhD)

Introduction: Microsurgical reconstruction of the male reproductive tract with vasooepididymostomy has been an effective method to resolve the epididymal obstruction in the obstructive azoospermia. Double-armed suture longitudinal intussusception vasooepididymostomy (DA-LIVE) has been widely adopted because of its relative simplicity and good success rates. Single-Armed Suture Longitudinal Intussusception Vasooepididymostomy (SA-LIVE), as an alternative method, allows the use of two single-armed sutures in situations in which double-armed sutures are not available, to perform microsurgical vasooepididymostomy. Here, a novel SA-LIVE was invented, named as Guo’s SA-LIVE, which could avoid the back-walling risks in situations when specialized double-armed microsutures were not available. With 2 flat overhand bends, only 2 single-armed sutures were used to perform Guo’s SA-LIVE procedure.

Methods: Male adult Wistar rats underwent vasectomy. Four weeks later, vasooepididymostomies were performed using DA-LIVE (n = 6), SA-LIVE (n = 6), or Guo’s SA-LIVE (n = 6) technique. After 12 weeks, patency was assessed functionally by evaluating for motile sperm distal to the anastomosis. If no motile sperm were visible, the mechanical defense system would substantially damage the sperm function and structure. High ROS levels noticed and indicative of compromised antioxidants defense system. This would lead to mitochondrial membrane leak and poor respiratory chain through deteriorated ATP production. The inability of the sperm cell or the epididymal system to overcome the excessive ROS insult produced by these abnormal sperm, thence leading to increase in the levels of LPO, DNA/chromosomal integrity damage in the hypercholeolesterolmic rats. Thus under hypercholesteroleic condition free radical mediated damage along with poor anti-oxidant defense system would substantially damage the sperm function and structure. These alterations were considerably reduced or recovered in hypercholesterolemic rats treated with M.pruilens. No adverse effects seen in control +M.pruilens.

Conclusion: These observations indicate the therapeutic efficacy of the M.pruilens seed extract towards controlling the multifaceted pathophysiological affecting sperm under hypercholesterolemia.

Funding: SERB, Govt. of India

To compare the Piwi-interacting RNAs (piRNAs) expression profile between unsuccessful and successful spermatozoa retrieval by micro-TESE in non-obstructive azoospermia patient testicular tissues. The differential expression levels of piRNAs were evaluated using small RNA-Seq method. Ontologic analyses were performed to determine the presence of enriched biological processes. Ten individuals with successful and unsuccessful sperm retrieval intron-obstructive azoospermia(NOA) underwent micro-TESE surgery were included with 5 cases in each group. The expression levels of 959 piRNAs were exhibited significant altered between successful and unsuccessful sperm retrieval groups. Those altered piRNAs were involved in many important biological pathways, including apoptosis, cell proliferation, and differentiation. A total of 18,324 homo sapiens piRNAs were identified by small RNA-Seq from NOA patient testicular tissues, among them 951 testicular piRNAs were significantly down regulated and 8 piRNAs were up-regulated in NOA patients with unsuccessful sperm retrieval groups (USRG) compared that of successful sperm retrieval groups (SSRG), respectively. 553 testicular piRNAs were found completely absent in USRG but showing abundant in SSRG. Testicular tissues from NOA patients with successful and unsuccessful spermatozoa retrieval exhibit a differential piRNAs profile. This provides new evidence that piRNAs have an essential role in spermatogenesis and may serve as novel non-invasive biomarkers for NOA patient diagnosis before assisted reproductive treatment in seminal plasma for further research.
Introduction: Modern sedentary lifestyle of many people with overwhelming stress, high calorie intake of nutritionally depleted food, smoking and alcohol consumption, triggers diseases like recurrent miscarriage, infertility, congenital malformations and childhood carcinomas. The transcriptionally inert spermatozoa delivers specific paternal transcripts responsible for embryonic development to the oocyte at fertilization and the dysregulation of these transcripts has been associated with increased incidence of recurrent pregnancy loss (RPL).

Objectives: The present study was designed to assess the effect of lifestyle factors on the levels of seminal oxidative stress, DNA damage and expression pattern of spermatozoal transcripts in male partners of couples experiencing RPL.

Methods: 75 male partners of couples experiencing RPL and 30 healthy volunteers with proven fertility were recruited for the study. Semen samples were obtained after 4 days of sexual abstinence and semen analysis was done by WHO (2010) criteria. Reactive oxygen species (ROS) levels were assessed by luminol-dependant chemiluminescence analysis was done by WHO (2010) criteria. Reactive oxygen species (ROS) levels were assessed by luminol-dependant chemiluminescence analysis. Gene expression was done by q-PCR and relative quantification (ROS) levels were assessed by luminol-dependant chemiluminescence (Presented By: Rima Dada, MD, PhD)

71 (Poster)
EFFECT OF LIFESTYLE FACTORS ON SPERMATOZOAAL HEALTH IN RECURRENT PREGNANCY LOSS
RIMA DADA MD, PhD¹, VIDHU DHAWAL MD², MANOJ KUMAR PhD³, DIPIKA DEKA MD², NEENA MALHOTRA MD², NEETA SINGH MD³ and VATSALA DADHWAL MD³
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ABSTRACTS
PD. However, few studies have explored drug-related PD risk factors. Here we examine the association between PD and drugs.

**Materials and Methods:** We analyzed subjects from the Truven Health MarketScan insurance claims database for years 2005, 2008, 2010, 2012, and 2014. Men with PD were identified using diagnosis (ICD-9) code, and were matched on age and number of clinic visits to control men without PD or ED. Association between PD, medications, and other diseases was assessed using logistic regression while adjusting for age, clinic visits per year, urologic visits per year, smoking, and obesity status. A Benjamin-Yekutieli adjustment was applied to decrease the false discovery rate.

**Results:** We included 35,555 men with PD and 355,550 controls, each group with an average age of 52.8±10.6 years. As a class, BB were associated with a decreased prevalence of PD (P < 0.0001). Cardedilol (OR, 95% CI 0.68, 0.66-0.71), metoprolol (0.79, 0.77-0.81), and atenolol (0.87, 0.84-0.89) followed this trend and were associated with decreased PD risk. Interestingly, propranolol (1.28, 1.22-1.35) was associated with increased risk for PD. When assessing other medications, an increased risk of PD was observed with antipsychotic and antidepressants medications, including lithium (1.73, 1.59-1.89), quetiapine (1.25, 1.18-1.32), bupropion (1.41, 1.37-1.44), venlafaxine (1.39, 1.34-1.44), and sertraline (1.27, 1.24-1.31). This trend tracked with medical diagnoses, as men with PD were at increased risk for psychiatric disorders (2.00, 1.68-2.38). An increased risk of PD was also observed with the antiviral drugs acyclovir (1.39, 1.35-1.44), valacyclovir (1.42, 1.38-1.46), and ritonavir (2.22, 1.94-2.55) correlating with an increased prevalence of HIV in men with PD (1.99, 1.84-2.14).

**Conclusion:** The risk of PD is variably associated with individual BB, with propranol having an increased risk of PD, and carvedilol, metoprolol, and atenolol having a decreased risk of PD. Antidepressants, antipsychotics, and antivirals are associated with an increased risk of PD and merit further investigation as potential PD risk factors.

**74 (Poster)**
**EPIDERMAL GROWTH FACTOR STIMULATES PROLIFERATION OF RAT STEM AND PROGENITOR LEYDIG CELLS VIA UPREGULATING CCND1**
Fei Ge MD¹, Yiyan Wang MD², Qingquan Lian PhD² and Ren-shan Ge PhD²
¹The Second Affiliated Hospital of Wenzhou Medical University; ²The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Fei Ge, MD)

**Introduction:** Epidermal growth factor (EGF) has many physiological roles. However, its effects on stem and progenitor Leydig cell development are still unclear. In this study, we examined the role of EGF in rat stem and progenitor Leydig cell development.

**Methods:** An established seminiferous tubule isolation and culture model with ethylene dimethanesulfonate (EDS) treatment to eliminate reproductive functions in infertile men diagnosed with major depressive disorder: A randomized controlled trial.
Madhuri Tolahunase MSc¹, Rajesh Sagar MD², Priyanka Chaurasia MSc¹ and Rima Dada MD, PhD³
¹Lab for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.; ²Department of Psychiatry, All India Institute of Medical Sciences, New Delhi, India. (Presented By: Madhuri R. Tolahunase, BAMS, MSc(Med))

**Background:** Infertility has a bidirectional relationship with depression. Neurodegeneration and regulatory feedback dysfunctions affect reproductive functions in infertile men diagnosed with major depressive disorder (MDD). Evidence suggests that drug treatment and ARTs have significant impact on health of the offspring.

**Objective:** To assess the evolution of semenogram, sperm quality and biomarkers of neuroplasticity and regulatory feedback systems in a sample of male infertile patients diagnosed with MDD during 12 weeks of yoga based lifestyle intervention (YBLI).

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Methods: In this 12–week RCT, male infertile patients (n=108) with MDD from a national apex institute in India were recruited and randomly assigned into either yoga group (n = 54), receiving YBLI or wound–list control group (n = 54). Primary outcomes were changes in semenogram [based on WHO (2010) guidelines], sperm quality (DNA fragmentation Index, reactive oxygen species and total anti–oxidant capacity), and biomarkers of neuroplasticity, and regulatory feedback systems (BDNF, serotonin, melatonin, cortisol, IL–6, DHEAS, and sirtuin 1). Secondary outcomes included changes in quality of life (WHOQOL–BREF scale), depression severity [(Beck Depression Inventory–II (BDI–II) scale), and systemic cellular health (DNA damage, oxidative stress, and telomere metabolism). The assessments were applied at 0, 4 and 12 weeks after the start of intervention.

Results: The results showed improvement in semen parameters, sperm quality and biomarkers of neuroplasticity and regulatory feedback systems during the 12 weeks, manifesting from the 4 week (all ANOVA, p<0.05). Likewise, statistically significant differences (all ANOVA, p<0.05) were observed showing increase in WHOQOL–BREF score, decrease in BDI–II scores, and improved cellular health biomarkers.

Conclusion: Yoga improved semen parameters and sperm quality by optimizing neuroplasticity and regulatory feedbacks in our sample of male infertile patients with MDD during 12 week intervention, in addition to improved quality of life, remission of depression and cellular health. Further research is needed to analyze the effects of yoga on infertile couples with MDD for benefits infertility.

Keywords: Male infertility; Depression, Neuroplasticity, Regulatory feedback; Yoga; Biomarker

77 (Poster)

IMPROVING REPRODUCTIVE EFFICIENCY IN THE BULL: WILL REDUCING ENDOGENOUS ESTRADIOL INCREASE SERTOLI CELL PROLIFERATION?

Kimberly Miller and Trish Berger PhD
Department of Animal Science
(Founded By: Kimberly Miller, BS)

Sertoli cells line the seminiferous tubules in the testes and normally proliferate prepuberally rather than postpuberally. Since Sertoli cells interact with germ cells during spermatogenesis, the number of Sertoli cells present at puberty directly determines the capacity to produce sperm postpuberally. Reducing testicular estradiol in male pigs prepuberally leads to an increase in Sertoli cell proliferation and number of Sertoli cells present postpuberally. This in turn increases testicular sperm production capacity, effectively making the boars “more fertile”. Increasing sperm reproductive capacity in dairy bulls would be advantageous to producers and consumers. The objective was to test the hypothesis that reducing testicular estradiol prepuberally in bulls would stimulate Sertoli cell proliferation and lead to increased numbers of Sertoli cells in the testis. Thirteen Jersey bull calves were treated with the same aromatase inhibitor, letrozole, used in previous studies with boars. At 26 weeks of age, testes were collected, weighed, fixed and Sertoli cell density determined. Sertoli cells were immunohistochemically labeled with GATA4 antibody. Testes were homogenized and testosterone and estradiol tissue concentrations determined. Aromatase gene expression was evaluated by qPCR. The dose of letrozole was twice the concentration determined to be effective in heifers, and testicular estradiol was decreased by one third (p < 0.05). However, the total number of Sertoli cells did not differ between letrozole-treated and vehicle-treated bulls. Testicular testosterone levels were not altered, nor was aromatase gene expression affected. While boars and bulls are both ungulates, the physiological levels of testicular estradiol are much lower (more than an order of magnitude) in bulls. Although the reduction in estradiol in letrozole-treated bulls was significant, concentrations are very low compared with concentrations in the boar. Hence, testicular estradiol may be too low to inhibit Sertoli cell proliferation in bulls. Alternatively, postnatal proliferation of Sertoli cells in bulls may correspond to the second wave of testicular sperm proliferation in bulls, which is not responsive to a reduction in endogenous estradiol. In conclusion, decreasing testicular estradiol postnatally with an aromatase inhibitor did not stimulate Sertoli cell proliferation in bull calves. (Supported in part by an American Jersey Cattle Association grant, UCD–FAFESP SPRINT, and MSP3171).

78 (Poster)

NOVEL ULTRA–PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY PROFILING OF ANDROGENOME IN HUMAN SALIVA

Nilesh Gaikwad PhD¹ and Prasanth Surampudi MD²
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(Presented By: Nilesh Gaikwad, PhD)

Introduction: Salivary Androgen measurements have the potential to provide a non–invasive assessment of androgenic steroids. The measurement of androgens in the saliva at such low levels is challenging for direct immunoassays which lack adequate accuracy. Mass spectrometry methods may help to overcome previous analytical limitations. In particular, a mass spectrometry method for human saliva that can provide multiple androgenic steroids involved androgen metabolism could provide a broader insight into potential alterations within the human body.

Methods: We recruited 75 healthy male and female human subjects ranging from 18 to 40 years of age and collected saliva sample. We developed and used a novel UPLC–MS/MS based platform for androgeneric profiling of saliva assay for simultaneous measurement of multiple androgens in saliva. The calibration curves for analysis exhibited linearity and reproducibility. The saliva samples were extracted with organic solvent, concentrated using speed–vac, and filtered before being introduced into a Waters Acquity UPLC system connected to a Xevo–TQ triple quadrupole mass spectrometer (UPLC–MS/MS). The resulting UPLC–MS/MS data was analyzed and processed using MassLynx 4.1 software.

Results: The UPLC–MS/MS results show the presence of many androgens in the human saliva. We were able to detect several androgens in the saliva including Androstenedione, Testosterone, 4-Androsten-3a-ol-17-one, 11-Ketotestosterone, Adrenosterone, 4-Androsten-3,6-17-trione, 7-KetoDHEA, 5a-androstane. Moreover, statistical comparison by Mann–Whitney U Test revealed that mean level of testosterone in saliva of males, 16.6 pg/ml, was significantly different (p=0.000005) than in females, 2.27 pg/ml. Similarly, level of 4-Androsten-3a-ol-17-one in men, 24.29pg/ml, was significantly different (p=0.03) than in females, 8.89pg/ml.

Conclusion: Salivary testing is desirable because of easy sample collection and could add to or potentially substitute serum testing of androgens in the future. Clinically, saliva collection has the potential to provide a high-throughput, non–invasive, and inexpensive technique to evaluate androgen levels. We have developed a novel method to better understand androgen metabolic pathways in human saliva. We propose that androgen alterations may be studied and monitored using the salivary androgenome profile. Analytical methods and results will be presented and discussed.

79 (Poster)

PORCINE PERIOVULATORY OVIDUCTAL FLUID INHIBITS THE CAMP/PKA PATHWAY DURING SPERM CAPACITATION IN MICE

Maria Gracia Gervasi PhD¹, Pablo Visconti PhD¹ and Carmen Matás DVM-PhD³
¹Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst; ²Department of Physiology, Faculty of Veterinary Science, International Excellence Campus for Higher Education and Research “Campus Mare Nostrum”, University of Murcia, Murcia, Spain; ³and Institute for Biomedical Research of Murcia, Murcia, Spain.
(Presented By: Maria Gracia Gervasi PhD)

Sperm capacitation is a process that involves the early activation of protein kinases (PKs) and inactivation of protein phosphatases (PPs). Phosphate activates the cAMP/PKA pathway, which induces an increase in tyrosine phosphorylation (pY). This entire process occurs in the female reproductive tract and various compounds present in the oviduct could have an important regulatory role during the capacitation process. We have previously shown in porcine that oviducal fluid from
periovulatory phase decreased PKA activity. However, it is not known if this inhibition is specie-specific. Thus, in this work we evaluated if periovulatory oviductal fluids from porcine have any influence in PKA activation and pY during sperm capacitation in mice. For this purpose, mice sperm was incubated for 1 hour in capacitating conditions either with or without follicular fluid (FF), oviductal fluid (OF) from the four phases of the estrus cycle (early follicular, late follicular, early luteal, and late luteal), and cumulus cells secretion media (CM). After treatment, sperm samples were resuspended in Laemmli buffer and separated on SDS-PAGE. The pattern of PKA-substrates phosphorylation and pY were evaluated by western blot. The results indicated that sperm incubated with FF, late luteal OF and CM have a similar PKA-substrates and pY pattern than sperm incubated in a capacitation media. However, periovulatory oviductal (late follicular and early luteal) fluids decrease the phosphorylation of PKA-substrates and pY during capacitation. A concentration-dependence was also observed. These results suggest that the periovulatory oviductal fluid influences sperm capacitation signaling, and that the inhibition of PKA by oviductal fluid is not specific to the species. Supported by MINECO-FEDER AGL2015-66341-R and Salvador de Madariaga (Ministerio de Educación Cultura y Deporte), and the Eunice Kennedy Shriver National Institute of Child Health and Human Development NIH grants R01HD38082 and R01HD44044.

80 (Poster)
CHANGES IN O-GLCNACYLATION DURING EPIDIDYMAL SPERM MATURATION
Maria Gracia Gervasi PhD, Darya Tourzani BS and Pablo Visconti PhD
Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst.
(Presented By: Maria Gracia Gervasi, PhD)

After leaving the testis, sperm undergo two sequential maturational processes before acquire fertilizing capacity: sperm maturation in the epididymis of the male, and sperm capacitation after ejaculation in the female reproductive tract. During transit through the epididymis sperm undergo several maturational changes, the acquisition of motility being one of them. The molecular basis of the regulation of this process are still not fully understood. Sperm are both transcriptionally and translationally silent, therefore post-translational modifications are key to regulate their function.

Contrary to capacitated mature sperm, immature sperm recovered from caput (first region of the epididymis) show no tyrosine phosphorylation after incubation in capacitating conditions. O-GlcNAcylation (O-Glc-NAc) has been shown to be a counterpart of phosphorylation in different cellular processes. Therefore, our work was aimed to characterize the O-Glc-NAc occurrence during sperm maturation.

Our results show that immature caput sperm expresses OGT, the enzyme responsible of O-Glc-NAc. Consistently, caput sperm present high levels of O-Glc-NAc when compared with cauda sperm. In addition, O-Glc-NAc proteins are localized in the sperm tail of immature sperm and are mostly absent in mature sperm. These results suggest that modulation of O-Glc-NAc occurs during sperm maturation. Altogether, our results serve as a starting point to study the regulation of the acquisition of sperm motility.
ABSTRACTS

TUESDAY, APRIL 24, 2018
*Poster Session III
11:15 a.m. - 12:15 p.m.
Location: The Nines Ballroom PreFunction
*Not CME Accredited

81 (Poster)
THE PSYCHOLOGICAL AND EMOTIONAL IMPACT OF SUB-FERTILITY ON MEN
Luriel Smith-Harrison MD, Abbey Kruper PsyD and Jay Sandlow MD
Medical College of Wisconsin
(Presented By: Luriel I. Smith-Harrison, MD)

Introduction/Objectives: Male-factor etiology is involved in approximately 50% of sub-fertile couples. While the medical work-up and management of this population is well-documented and studied, the impact of sub-fertility on the emotional and psychological well-being of these men has not been studied in depth. This study aims to quantify the psychological impact and resource needs of men with sub-fertility.

Methods: Upon arrival at a single, academic Reproductive Medicine Center, all men being evaluated for sub-fertility were provided with a qualitative questionnaire. Several psychologic and emotional domains were probed with Likert scales. These domains included effect on mood, marital relationship, and sexual experience. Further Likert scales were used to better characterize the participants’ abilities to cope with sub-fertility. All data was analyzed in standard, statistical fashion.

Results: Fifty-eight men were able to complete the questionnaire. Twenty-three of 58 men (39%) reported a negative effect on their mood. In addition, 21% reported a negative effect on their marriage, while 10% of men reported a negative impact on their sexual experience. While the majority of men felt that they were able to cope, nearly 25% of men had doubts about their ability to manage the emotional toll. Additionally, 25% of men requested additional resources to address the emotional and behavioral effects of sub-fertility.

Conclusion: Sub-fertility has a significant impact on the emotional and psychological well-being of men who present at reproductive clinics. Up to 25% of men feel the need for further emotional and behavioral support. While the medical management of these patients is paramount, the psychological ramifications must also be addressed to improve patient quality of care and treatment outcomes.

82 (Poster)
THE OPTIMAL SURGICAL TIMING FOR FOURNIER’S GANGRENE
Ta-Yao Tai MD, Tsung-Yen Lin MD and Yung-Ming Lin PhD
National Cheng Kung University Hospital
(Presented By: Ta-Yao Tai, MD)

Purpose: Fournier’s gangrene (FG) is known to be a life-threatening disease with mortality rates ranging from 7.5 to 45%. Given the high mortality rate in those patients, emergent surgical intervention is always needed. This study is focused on the determination the optimal timing for surgical intervention, which might provide better prognosis in this disease entity.

Materials and Methods: From 1979 to 2016, a total of 100 patients diagnosed with Fournier’s gangrene in National Cheng Kung University Hospital were retrospectively reviewed. Patients’ demographics, laboratory parameters at initial diagnosis, Fournier’s gangrene severity index (FGSI) and simplified FGSI, the time interval between the time of arriving emergency room (ER) and the time of surgical intervention (OR) were recorded. All of the patients received aggressive surgical intervention. The patients were divided into survival and non-survival groups, and the time interval to surgical intervention between the two groups was analyzed. Youden index was used to subdivide our patients into early and delayed intervention groups. All parameters were also compared between the two groups.

Results: Based on simplified FGSI, the mortality rate was 0% in score 0, 0% in score 1, 6.67% in score 2, 35.71% in score 3, 25% in score 4, 40% in score 5, 60% in score 6, 100% in score >7. Thirty-seven patients with the score between 3 and 6 were further investigated. The mean time interval between ER and OR in survivors (7.3 ± 6.2 x 100 mins) was significantly lower than non-survivors (13.9 ± 8.9 x 100 mins) (P=0.037). Then we defined 15 hours as cut-off time interval and those 30 patients were subdivided into early (n = 21) and delayed (n = 16) groups. Basic characteristics, laboratory parameter at initial diagnosis, FGSI and simplified FGSI were not significantly different between these two groups. The mortality rate was significantly lower in early intervention group (23.8%) compared to delayed group (68.8%) (P=0.034).

Conclusion: Early surgical intervention within 15 hours can significantly decrease mortality rate in high-risk patients with Fournier’s gangrene.

83 (Poster)
METABOLIC PROFILE OF GENDER DYSPHORIC PERSONS IN A LONG-TERM TREATMENT WITH CROSSEX HORMONES
Stella Santiago MD¹, Henrique Cecotti MD², Flavia Cunha Doctor³, Sorahia Domenice Doctor¹, Berenice Mendonca Professor¹ and Elaine Costa MD, PhD, Prof²
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Background: Crosssex hormone therapy (CSHT) is essential of gender affirming treatment for gender dysphoric persons. To date, the effect of CSHT on the components of metabolic syndrome remains incompletely elucidated.

Objective: to evaluate the impact of long-term CSHT on metabolic parameters of both transgender women and transgender men. Patients and Methods: a crosssectional study of 139 trans women with mean age of 35 years (19-56 yrs) and 42 trans men with mean age of 42 years (25-61yrs) followed at our outpatient clinic from 1998 to 2015. We evaluated BMI, blood pressure, fasting glycaemia and insulinemia, hemoglobin A1C and lipid profile. At the first visit, 88% of trans women referred unsupervised use of several preparations of estrogens and/or progestagens and 45% of trans men referred previous use of testosterone preparations. At that time, oral conjugated equine estrogens (CEE) at doses of 0,625 or 1,25 mg/day associated with ciproterone acetate (CA) at doses of 50 or 100 mg/day was prescribed for the majority of the trans women (29 subjects received CEE alone). For trans men, the short-acting testosterone cypionate at dose of 200 mg every 15 days was prescribed for almost all of them (7 patients used long-acting testosterone undecanoate, 1000 mg every 12 weeks) at first visit. The average time of hormonal treatment was 10 yrs in both groups.

Results: trans women with combined therapy (CEE plus CA) showed lower HDL levels than the CEE alone group (57.4±21 versus 46.7±15 mg/dL; p=0.02) as well as fasting glycaemia levels (92.5±14 versus 86.9±9.1 mg/dL; p=0.03). On the other hand, the remaining variables were similar in both groups. Trans men patients using testosterone cypionate showed higher triglyceride levels than those with long-acting testosterone (137±90 versus 72±39 mg/dL; p=0.008), likewise for fasting glycaemia levels (93±13 versus 78±10 mg/dL) and BMI (28±5 versus 24±4; p = 0.03). Regarding other variables, no differences were observed.

Conclusion: Our data suggest that crosssex hormone long-term treatment is safe to metabolic parameters when doses of hypogonadal patients’ replacement therapy were used. However, regarding fasting glucose levels, CA and short-acting testosterone cypionate was detrimental. Additionally, CA and short-acting testosterone cypionate negatively affected HDL and triglyceride levels, respectively.
ABSTRACTS

84 (Poster)
MICROSATELLITE INSTABILITY IN SELECTED GENES IMPLICATED IN MALE INFERTILITY
Jyoti Sharma M Tech¹, Prakash Chand Sharma M Sc, PhD², Rohit Kumar Khetan M Sc¹, Priyanka Chaurasia M Sc¹, Rajeev Kumar MD³ and Rima Dada MD, PhD³
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(Presented By: Jyoti Sharma, M.Tech)

Introduction: Microsatellites (MS) are tandemly repeated short DNA motifs. Being hypervariable, microsatellites show length polymorphism termed as Microsatellite Instability (MSI), a diagnostic phenotype of various carcinomas, particularly colon cancer, and a significant prognostic marker of testicular tumors of germ cells. An earlier solitary study evaluated implication of MSI in azoospermic men. Considering a significant increase in the incidences of male infertility worldwide, this study was undertaken to analyze length polymorphism at MS regions in three genes implicated in male infertility.

Methods: Seventy five male infertility related genes were screened for microsatellites using MISA, a MicroSatellite search tool. Five microsatellite motifs in three genes (Androgen Receptor-AR, Calmodulin Kinase-CAMK4 and Glial cell Derived Neurotrophic Factor-GDNF) were selected to detect MS length polymorphism. Blood and semen samples were collected from 10 each of the normozoospermic, oligozoospermic, azoospermic cases of primary infertility and healthy controls. The PCR amplicons from blood and sperm DNA were resolved on polyacrylamide gels.

Results: (CAG)n motif of AR gene showed maximum polymorphism while its (GGC)n motif was monomorphic. MS motif of GDNF gene showed least polymorphism compared to that of AR and CAMK4 genes. In normozoospermic cases, an additional allele of MS loci of GDNF gene was found in sperm DNA only suggesting a possibility of mutation at this locus during spermatogenesis. Highest level of heterozygosity was found in MS loci of CAMK4 gene.

Conclusion: Length polymorphism observed at different MS regions of these genes suggest occurrence of some mutational events like insertions or deletions which require further validation by sequence analysis. Moreover, a possible correlation between oxidative stress and MSI needs investigation for better understanding of molecular basis of male infertility and to assess whether MSI is a potential link between infertility and cancer.

85 (Poster) - WITHDRAWN

86 (Poster)
TESTOSTERONE, VITAMIN D, NUTRITIONAL AND METABOLIC STATUS. IS THERE A LINK AMONG THEM?
Inari Ciccone MSc Student¹, Juliana Pariz MD, PH¹, Elaine Costa MD, PHD² and Jorge Hallak MD, PhD²
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(Presented By: Inari Ciccone, Msc Student)

Background: Recent studies have demonstrated that obesity and metabolic syndrome are associated with low serum testosterone, but the direction and causality of this relationship is not completely understood. Moreover, the link of vitamin D with testicular function and sexual hormones have been investigated.

Objective: To evaluate the effect of vitamin D serum levels metabolic and nutritional status on testosterone concentrations in male subjects.

Patients and Methods: We selected 260 men (aged 18 to 60 y.o.) from a private andrology reference medical clinic. Total and free testosterone, 25(OH) vitamin D serum concentration, HOMA-IR, BMI and total cholesterol were analyzed. Lifestyle factors such as smoking, alcohol and sedentary were also considered, as well as age and presence of varicocele. Statistical analysis were performed by SPSS program version 19.0 (SPSS Inc. Chicago, IL). For non-parametric variables. The Spearman coefficient correlation and Mann-Whitney association tests were applied. Then, for variables with statistical significance, a regression model was performed. Statistical significance was considered with P value < 0.05.

Results: We found a significant positive correlation between total testosterone and vitamin D levels (p=0.014). Total testosterone levels were negatively correlated with BMI, HOMA-IR and age (table1). No correlation was found among testosterone and the remaining parameters.

Conclusion: We demonstrated a strong correlation between vitamin D levels and testosterone concentration, supporting the relevance of vitamin D on testis function. Our data also confirmed the negative influence of overweight, insulin resistance and age on testosterone levels.

87 (Poster)
THE ASSOCIATION OF A HISTORY OF SEXUALLY TRANSMITTED INFECTIONS WITH SEMEN ANALYSIS PARAMETERS IN THE INFERTILE PATIENT
Nahid Punjani MD, MPH¹, Madhur Nayan MD, CM, PhD², Ethan Grober MD, MED, FRCSC³, Kirk Lo MD, CM, FRCSC³, Susan Lau BSc² and Keith Jarvi MD, FRCSC³
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(Presented By: Nahid Punjani, MD, MPH)

Introduction and Objective: From our previous studies, we have shown that laboratory documented genitourinary sexually transmitted infections (STIs) are uncommon in our population (Chlamydia trachomatis (CT) 0.3% and Neisseria gonorrhea (GC) 0.05%) and in our series these STIs were not associated with abnormal semen parameters. The association of a previous history of STIs with semen parameters is not well known. Our objective was to correlate a history of sexual transmitted infections to semen parameters in men presenting with infertility.

Methods: Data from all men between 2008 and 2017 presenting for male infertility assessment was prospectively collected and retrospectively reviewed at a single institution. Self-reported questionnaires were used to collect demographic data and clinical history. Using the 2010 World Health Organization criteria, examined semen analysis parameters included volume, count, morphology, motility and vitality. Anti-sperm antibodies were considered if either sperm IgA or IgG was detected. Self-reported history of GC, CT, syphilis, herpes and genital warts was utilized.

Results: A total of 9079 patients were reviewed, of which 5,526 (61%) patients had documented semen analysis data. Of these 149 (2.7%) had reported GC, 284 (5.1%) had reported CT, 19 (0.3%) had reported syphilis, 178 (3.2%) had reported herpes and 228 (4.1%) had reported genital warts. Patients with history of GC were more likely to be oligospermic (OR 2.08, p<0.01) and have decreased volume (OR 1.60, p=0.04). Patients with history of genital warts were more likely to possess

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Introduction and Objective: The Androgen Deficiency of the Aging Male (ADAM) questionnaire is a clinically utilized screening questionnaire for male hypogonadism. Our objective was to correlate hypogonadism as diagnosed by ADAM scores to semen parameters in men with infertility.

Methods: Between 2008 and 2017, data from all men at a single institution presenting for infertility assessment was prospectively collected and retrospectively reviewed. Clinical history and relevant demographic data was obtained through patient self-reported questionnaires. Collected semen analysis parameters included volume, count, morphology, motility and vitality, and appropriate cut-offs were compared to the 2010 World Health Organization values. Baseline testosterone level was recorded. ADAM questionnaire data was recorded as ten separate questions, and a positive ADAM test was considered as a single question. Analysis was completed for all patients with available data.

Results: A total of 9079 patients were reviewed, of which 5,526 (61%) patients had documented semen analysis and ADAM questionnaire data. As expected, a positive ADAM test correlated with total testosterone (<10nmol/l) levels in keeping with hypogonadism (p<0.05). Average testosterone levels in negative screening men was 13.4 nmol/l and in positive screening men was 12.8 nmol/l. After adjusting for age and total testosterone, patients with a positive ADAM test were less likely to be azoospermic (OR 0.83, p<0.03), oligospermic (OR 0.77, p<0.01), or have poor sperm motility (OR 0.72, p<0.01). However, those with positive ADAM test were more likely to have lower ejaculate volume (OR 1.45, p<0.01). There was no effect seen on sperm motility or vitality.

Conclusion: Our study illustrates that the hypogonadism as defined by the ADAM test does correlate with various semen parameters. This questionnaire may be used as an adjunctive measure in the work-up and management of infertile men.

89 (Poster) - WITHDRAWN

90 (Poster)

IS THERE A RELATIONSHIP BETWEEN INCREASING PATERNAL AGE, SPERM DNA DAMAGE, AND OXIDATIVE STRESS IN INFERTILE MEN?

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(Presented By: Andrew Lee, BSc)

Introduction: As delaying parenthood is becoming more prevalent in the 21st century, it is necessary to understand how age impacts fertility. Several studies have corroborated a relationship between advancing paternal age and deteriorating sperm quality. Specifically, it is known that sperm DNA damage increases with male age and may result in increased miscarriage rates and a higher frequency of genetic disorders in offspring. Despite sperm oxidative stress playing a key role in the etiology of sperm DNA damage, a relationship between oxidative stress and increasing paternal age has not yet been well-established. The objective of this study is to analyze the relationship between male age and oxidative stress in an infertile population.

Methods: Excess, donated ejaculates from male patients after 2-5 days of abstinence were used for this institutional REB approved study. Sperm DNA damage was evaluated by standard flow cytometric acridine orange-based assay and reported as DNA Fragmentation Index (DFI). Using the MiOXSYS system, sperm oxidative stress was evaluated within the same population as static oxidation reduction potential (sORP), an integrated measure of the balance between total oxidant activity to total reductant activity. The relationship between DFI and paternal age, sORP and paternal age, and DFI and sORP were determined.

Results: The mean paternal age was 38.4±5.7 years (age range was 29.6-52.4 years). There was a positive correlation between DFI and paternal age approaching statistical significance (r=0.27, p=0.09, n=39). In the same population, there was no correlation between paternal age and sORP (r=0.07, p=0.67, n=42). However, there was a strong positive correlation between sORP and DFI (r=0.45, p=0.005, n=39).

Conclusion: We confirm that there is a positive correlation between male age and increased DNA damage. However, sperm oxidative stress (sORP) was not correlated to paternal age in the same patient population. Though oxidative stress is a major cause of DNA damage, it could be that the age-related increases in sperm DNA damage are mediated by other factors such as aberrant chromatin compaction and apoptosis. This is consistent with our additional finding that oxidative stress is positively correlated to sperm DNA damage, despite a lack of correlation with paternal age.

91 (Poster)

INDUCED PLURIPOTENT STEM CELL-DERIVED CONDITIONAL MEDIUM PROMOTES LEYDIG CELL ANTI-APOPTOSIS AND PROLIFERATION VIA AUTOHAPHY AND WNT/B-CATENIN PATHWAY

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(Presented By: Xiaoling Guo)

Leydig cell transplantation could be a better alternative in treatment androgen-deficient males. The main purpose of this study was to investigate the effects of induced pluripotent stem cell-derived conditioned medium (iPS-CM) on the anti-apoptosis, proliferation, and function of immature Leydig cells (ILCs), and illuminate the underlying mechanisms. ILCs were exposed to 200 µM hydrogen peroxide (H2O2) for 24 h with or
ABSTRACTS

92 (Poster)
TIME SPENT WALKING AND SITTING: IS THERE AN ASSOCIATION WITH SEMEN PARAMETERS?
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(Presented By: Justin Houman, MD)

Introduction: Sedentary lifestyle is a risk factor for cancer, cardiovascular disease, diabetes and other health outcomes, including adverse effects on semen parameters. Because sitting and sedentary lifestyle are modifiable behaviors, men may benefit from knowing how these behaviors potentially affect their fertility.

Objective: To investigate associations between semen parameters, time spent sitting, and physical activity in a clinical population with male factor infertility.

Methods: Participants were enrolled at the male infertility clinic at Cedars-Sinai Medical Center. Data included history, physical exam, laboratory tests collected during clinical care and research data collected using the International Index of Erectile Function, International Physical Activity Questionnaire, blood and semen.

Results: Study participants (n=46) ranged in age from 27 to 61 years (39.4 ± 7.1), BMI 20.1 to 46.9 (27.2 ± 4.7). Average duration sitting each day (total of leisure, work, commuting) was 7.5 hours and walking (>10 minutes per occurrence) averaged 1.2 hours per day. The mean and standard deviation for sperm concentration was 42.1 ± 32.1 million per ml; sperm motility 30.7 ± 24.4 percent. Job titles of participants spanned 9 of the 10 National Occupational Research Agenda Sector Programs but were not associated with semen parameters in this data. Pairwise correlations indicated a negative association between time sitting during commuting in motor vehicles and total motile sperm, r(43)= -0.30, p=0.04; and a positive correlation between leisure time walking and sperm concentration above 15 million per ml, r(39)=0.57, p=0.05. Men who reported any leisure time walking were 2X more likely to have sperm motility >40% and 3X more likely to have sperm concentration above 15 million per ml.

Conclusion: Correlation between sitting while commuting in motor vehicles and sperm motility is a new finding that deserves further attention. Other correlations in the data showed negative associations between sperm parameters and longer sitting times, and positive associations with longer walking times but did not reach statistical significance. We continue to enroll participants. Male infertility has numerous etiological factors but growing evidence indicates that time that a man spends inactive or sitting during work or leisure might negatively impact fertility.

93 (Poster)
CLASSICAL VERSUS NON-CLASSICAL HYPOGONADISM
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(Presented By: Noah Vincenzo Lupica)

Introduction and Objective: In 2015, the FDA limited the indications for treatment of men with symptomatic androgen deficiency to men with “classical hypogonadism,” where an identifiable underlying cause, such as Klinefelter’s or prior chemotherapy, is present. In clinical practice, the majority do not fit this classical criterion and represent “non-classical hypogonadism.” This “non-classical” population is yet to be characterized, leaving clinicians without established guidelines for their treatment. We hypothesized that adult patients with classical and non-classical hypogonadism would present with similar demographic and baseline testosterone levels.

Methods: We obtained a list of all patients evaluated at the Men’s Health Clinic of the Miriam Hospital between 01/01/2008 – 10/01/2016 who fit the following criteria: male sex, at least 18 years of age, ICD-9 diagnosis of hypogonadotropic hypogonadism (253.4), other testicular hypofunction (257.2), or Klinefelter’s Syndrome (758.7). If an identifiable etiology was found in the diagnosis code or clinical notes, the patient was recorded as having “classical hypogonadism”; otherwise, the patient was recorded as having “non-classical hypogonadism.” Age, body mass index (BMI), baseline laboratory hormone values were recorded and compared across groups.

Results: Of the 107 patients who met our inclusion criteria, 8 patients (2.5%) fit the definition of classical hypogonadism, while the other 99 (92.5%) patients were considered non-classical cases. A Wilcoxon Rank Sum Test showed no statistically significant difference along the parameters of age, BMI, baseline testosterone level and prolactin level. There was a significant difference in luteinizing hormone (LH) and follicular stimulating hormone (FSH) levels between the classical and non-classical groups (see figure).

Conclusion: The vast majority of hypogonadism cases seen in our clinic fall into the “non-classical” category. Although LH and FSH levels differ between the two groups due to underlying pituitary pathologies in the “classical hypogonadism” group, the basic demographic values such as age and BMI, as well as the baseline testosterone levels, are not significantly different.

94 (Poster)
EFFECTS OF CROSS-SEX HORMONE THERAPY ON TESTICULAR MORPHOLOGY IN TRANS WOMEN AT TRANSSEXUAL PROGRAM IN A REFERRAL CENTER FOR ENDOCRINOLOGY IN BRAZIL
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(Presented By: Elaine Frade Costa, MD, PhD, Prof)

Elevated peripheral estrogen levels have diverse effects on male reproductive organs causing gonadotropin and spermatogenic suppression. Testicular atrophy, follicular hialinization and decreased spermatogenesis are some possible effects of this therapy. However, it has been noticed a highly heterogeneous findings in tests that can be explained by insufficient cross-sex hormone therapy regarding dosage or duration. To understand the effect of cross-sex hormone treatment on testicular histology in trans women, we accomplished a retrospective study including 40 patients that were submitted to sex-reassignment surgery (SRS) between 2001 to 2017 at Hospital das Clínicas of University of São Paulo. Age, time

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of estrogen before SRS, Cyproterone acetate use, Gonadotropin, serum estradiol and testosterone before SRS were collected. Estrogen conjugate 0.625 mg was the estrogen available in our department. Testicular tissues were obtained on the day of SRS. Material received and fixed in formalin, stained with hematoxylin and eosin. Laminae were analyzies by available pathologist at that time and these data were collected in our database. We assessed atrophy of testicular parenchyma, tubular hylanization and the degree of spermatogenesis. Results: The mean duration of estrogen therapy was 14.8 years 3.5, and cyproterone was employed in 70.0% of patients. Mean hormone dosage levels were: FSH (IU/L) 4.72.3, LH (IU/L) 9.14.5, estradiol (pg/mL) 42.4 7.1 and total testosterone (ng/dL) 95.2 44.2. Testicular atrophy was the most prevalent histologic feature (prevalence of 80.0%12.4%), followed by decreased or absent spermatogenesis (60.0%15.2%) and follicular hylanization (15.0%11.1%). Presence of these findings were independent of estrogen therapy duration and of cyproterone use in our cohort according to Chi-square test, although the prevalence of impaired spermatogenesis was slightly higher in patients in use of cyproterone (67.9%17.3%) compared to patients without use of cyproterone (41.7%27.9%), but not statistically significant (p = 0.121). Only one patient presented cavernous body fibrosis. Conclusion: The use of cross-sex hormone treatment is not associated to testicular malignant degeneration. Moreover, testicular atrophy and impaired spermatogenesis are common findings in trans-women. Expanding the studied cohort will better characterize the impact of cyproterone on spermatogenesis.

95 (Poster)
ONCOSTATIN M INHIBITS DIFFERENTIATION OF RAT STEM LEYDIG CELLS IN VIVO AND IN VITRO
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(Presented By: Renshan Ge, MD, MS)

Oncostatin M (OSM) is a pleiotropic cytokine within the IL-6 family of cytokines which regulates cell growth and differentiation in a wide variety of biological systems, including hematopoiesis, neurogenesis, and osteogenesis. However, its action and underlying mechanisms on stem Leydig cell is unclear. The objective of the present study was to investigate whether OSM affects the proliferation and differentiation of rat stem Leydig cells. In order to quest the effects of OSM on stem Leydig cell development, we used a unique in vitro system of cultured seminiferous tubules with ethane dimethane sulfonate (EDS) and Leydig cell-depleted rat testis in vivo to assess the ability of OSM to regulate the proliferation and differentiation of rat stem Leydig cells. Intratesticular injection of OSM (10 and 100 ng/testis) blocked the regeneration of Leydig cells by reducing the serum testosterone level and Leydig cell-specific gene (Lhcgr, Star, Cyp11a1, Hsd3b1, Cyp17a1, and Hsd11b1) and their protein expression levels. OSM has no effect on serum luteinizing hormone and follicle-stimulating hormone levels as well as the proliferative capacity of Leydig cells in vivo. As for the effects of OSM in vitro, OSM (0.1, 1, 10, and 100 ng/ml) inhibited the differentiation of stem Leydig cells by reducing medium testosterone levels and down-regulating Leydig cell-specific gene (Lhcgr, Star, Cyp11a1, Hsd3b1, Cyp17a1, and Hsd11b1) and their protein expression levels. Taken together, the results of the present study suggest that OSM is an inhibitory factor of stem Leydig cell development. Keywords: Oncostatin M; Stem Leydig cells; differentiation; Testosterone. The present study was supported by NSFC (81730042 to R.S.G.) and Zhejiang Provincial NSFC (LY15H310008 to R.S.G.).

96 (Poster)
COMBINED USE OF GM1 LOCALIZATION PATTERNS AND SEMEN ANALYSIS PARAMETERS TO IDENTIFY INDIVIDUALS WITH HIGHER OR LOWER CHANGES OF ACHIEVING PREGNANCY
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(Presented By: Eric K. Seaman, MD)

Introduction and Objective: Semen analysis is limited in its ability to predict fertility in part due to its lack of assessment of sperm function. Cap-Score™, a validated sperm function test, uses GM1 localization patterns to measure capacitation. Sperm must capacitate in order to fertilize. The purpose of this study was to determine if semen analysis measures, together with Cap-Score, could effectively assess the ability of men who to initiate a pregnancy.

Methods: Cap-Score™ and standard semen analysis were performed on two populations of men: 1) men seeking fertility assistance from a reproductive endocrinologist (n=58; completed three rounds of IUI or achieved pregnancy; Pop1), and 2) men seeking fertility assistance from a reproductive urologist (n=23; still trying to conceive or having already achieved pregnancy; Pop2). Linear discriminant analysis (LDA) of Pop1 determined the best combination of Cap-Score and semen analysis parameters to separate men who were successful (P) versus unsuccessful (NP; p=0.049). Men were defined as “successful” if they conceived through either natural means or in ≤ 3 rounds of IUI. The resulting linear combination was then applied prospectively to predict outcomes in Pop2, as obtained from an evaluation follow-up questionnaire. For those in Pop2 that achieved pregnancy, the length of time between seeing the Urologist and questionnaire ranged from 3.4 to 8.9 months while those who were still trying ranged 4.0 to 9.7 months.

Results: 9 individuals in Pop2 successfully generated a pregnancy and 14 are still trying to conceive. 72% (5/7) of the individuals from Pop2 that were placed into the NP group have not yet generated a pregnancy. Furthermore, 78% (7/9) of the individuals attaining pregnancy were correctly predicted. P. The P group (n=16) was further subdivided into “high” and “mid” groups to maximize the proportion of P. 63% (5/8) and 25% (2/8) of the individuals placed in the high and mid groups achieved pregnancy. The proportion of successful individuals in the high group was greater than the generally accepted success rate of 29% for stimulated IUI with 3 cycles (p=0.013).

Conclusion: These data indicate that a combination of Cap-Score and semen analysis provides clinically-relevant information concerning male fertility. Knowing if an individual is in the high, mid or low group will allow clinicians to choose the most appropriate fertility treatment.

97 (Poster)
INCREASED RISK OF CANCER IN MEN WITH PEYRONIE’S DISEASE
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(Presented By: Taylor P. Kohn, M.Phil.)

Objective: Men with Peyronie’s Disease (PD) may have increased prevalent comorbidities, including malignancy. Here we examine the relationship between PD and malignancy using clinical and genetic data.

Materials and Methods: We analyzed data from the Truven Health MarketScan claims database from 2007-2013. Men with PD were compared to men matched for age and duration of follow-up with erectile dysfunction (ED) and controls. The incidence of the categories of malignancy was compared among each group utilizing a Cox regression model. A father and son with both PD and Dupuytren’s disease (DD), a related fibrotic diathesis, were whole exome sequenced (WES), and shared non-synonymous single nucleotide polymorphisms (SNPs), which were
analyzed for association with malignancy, and cross-referenced against gene expression data from The Cancer Genome Atlas (TCGA).

Results: In all, 48,423 men with PD, 1,177,428 men with ED, and 484,230 controls were identified. The mean age among all three cohorts was ~49 years old, and mean follow-up time was ~4.4 years. Compared to controls, men with PD had an increased risk of stomach cancer (HR, 95% CI; 1.43, 1.06-1.14) testis cancer (1.39, 1.05-1.84), and melanoma (1.19, 1.02-1.38). When compared to men with ED, men with PD had an increased risk of developing prostate cancer (1.38, 1.28-1.49). Analysis of WES data identified 150 shared, potentially deleterious nonsynonymous SNPs overlapping with altered genes involved in genitourinary and gastrointestinal cancers. Using TCGA, expression data demonstrated that these techniques may be considered as standard part of the cryopreservation protocol in patients who seek fertility preservation.

Conclusion: Men with PD are at increased risk for numerous malignancies compared to age matched men with ED and controls. Genetic alterations in men with both PD and DD independently support a risk for genitourinary and gastrointestinal cancers. These findings suggest that men with PD should be closely monitored after diagnosis (and treatment) of their PD.

98 (Poster)
IMPROVING POST-THAW SPERM CRYOSURVIVAL RATES IN THE ANDROLOGY LAB: CHOOSING THE BEST PROCESSING TECHNIQUE PREVIOUS TO THE CRYOPREervation IN ACCORDING TO INITIAL SPERM CHARACTERISTICS
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Introduction: Seminal processing before cryopreservation is not always used, but depending on initial semen characteristics may be crucial to improve post-thaw cryosurvival rates.

Objective: To determine the optimal pre-freeze semen processing method to improve post-thaw sperm quality in according to initial semen characteristics.

Methods: Ninety-one normozoospermic (NOR) and 181 asthenozoospermic (AST) men (19 to 68 y.o.) had semen samples cryopreserved from 2002-2017. After routine initial analyses, samples were processed by three different methods: simple centrifugation, density gradient using Isolate® or simple washing and were subsequently cryopreserved with Test Yolk Buffer (TYB) + glycerol 6% and serum substitute (SSS) by the liquid vapor freezing method. After 24 hours, a small sample was evaluated. The one-way ANOVA test was used for statistical analysis and adopted p<0.05.

Results: TYB samples were used as control group, with total motility NOR=72.82±8.64% AST=47.21±19.79%, progressive motility NOR=53.51±12.93% AST=15.77±10.45%, and cryosurvival (CS) NOR=18.29±16.64% AST=53.51±12.93% in NOR group, the association of density gradient processing and TYB + SSS showed an increase in grade B motility (49.33±11.19%, p=0.013) and in CS (36.51±31.42%, p<0.009) when compared to control group. When evaluating the simple wash + SSS, a reduction of total motility (62.00±5.70%, p=0.0001), progressive motility (43.00±15.24%, p=0.004), grade B motility (35.00±16.20%, p=0.027), and in CS (8.10±6.59%, p=0.037) was observed. In AST group, the addition of TYB + SSS increased sample CS (37.68±33.03; p<0.001) when compared to control group.

Conclusion: We conclude that the density gradient pre-freeze seminal processing with TYB + SSS increased in the PD men post-thaw sperm quality in normozoospermic samples. In addition, the use of SSS improved post-thaw sperm quality in asthenozoospermic samples. We suggest that these techniques may be considered as standard part of the cryopreservation protocol in patients who seek fertility preservation.

99 (Poster)
LONG-TERM TESTOSTERONE THERAPY (TTh) WITH TESTOSTERONE UNDECANATE INJECTIONS (TU) COMPLETELY PREVENTS PROGRESSION FROM PREDIABETES TO TYPE 2 DIABETES (T2DM) IN 45 HYPOGONADAL MEN WITH PREDIABETES: REAL-LIFE DATA FROM A REGISTRY STUDY
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Introduction and Objectives: The lifetime risk to progress from prediabetes to diabetes was recently reported as 74.0%. The prevention strategy is weight loss. Since long-term TTh in hypogonadal men results in sustained weight loss, we studied hypogonadal men with prediabetes defined by HbA1c of 5.7-6.4%.

Methods: Of 400 men with symptomatic hypogonadism (total testosterone ≤12.1 nmol/L), 45 (11.3%) had prediabetes and had presented to the urology office with erectile or urinary function complaints. All men received TU 1000 mg/12 weeks (T-group) following an initial 6-week interval. No structured recommendations for lifestyle modifications were provided. Measurements were performed 2 to 4 times a year for up to 11 years. 9-year data are presented.

Results: Mean age: 55.2±8.0. 91% years. Mean HbA1c decreased from 5.9±0.2 to 5.2±0.2% after 9 years (p<0.0001), fasting glucose from 5.5±0.2 to 5.3±0.1 mmol/L (p<0.05). Surrogate markers of insulin resistance: triglyceride/HDL ratio decreased from 7.3±1.8 to 3.5±0.7, TyG index, also a predictor for the risk of incident diabetes, from 9.5±0.2 to 9.1±0.1 (p<0.0001 for both). Weight dropped from 99.7±14.2 to 85.1±14.7 kg (p<0.0001), waist circumference from 102.4±6.7 to 94.5±5.6 cm, BMI from 32.0±4.8 to 27.7±2.9 kg/m2, weight change from baseline -17.9% (p<0.0001 for all). No patient progressed from prediabetes to T2DM during the entire observation period. 44 patients’ last measured HbA1c was <5.7% which is below the prediabetes range. Only 1 patient’s last HbA1c was 6.0%.

Conclusion: Long-term TTh with TU in hypogonadal men with prediabetes completely prevented progression from prediabetes to T2DM.

100 (Poster)
REDUCTION OF OBESITY, MORTALITY AND MAJOR ADVERSE CARDIOVASCULAR EVENTS (MACE) IN HYPOGONADAL MEN WITH TYPE 2 DIABETES (T2DM) RECEIVING LONG-TERM TESTOSTERONE THERAPY (TTh): REAL-LIFE DATA FROM A REGISTRY STUDY
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1Bayer AG, Berlin, Germany; 2Gulf Medical University, Ajman, UAE; 3Private Urology Practise; 4Boston University School of Public Health; 5Boston University School of Medicine

Introduction and Objectives: Obesity and T2DM are highly prevalent in men with hypogonadism and vice versa. Methods: In a registry of 776 men with testosterone (T) ≤12.1 nmol/L and hypogonadal symptoms, 286 (36.9%) had T2DM. 133 received TU 1000 mg/12 weeks (T-group), 153 had opted against TTh and served as controls (CTRL).10-year data were analyzed.

Results: Mean age: 61.8±5.4 years (T-group), 64.2±4.6 (CTRL). 91% in the T-group and 72% in CTRL were obese. In the T-group, 18% had excesses obesity, defined by BMI ≥30 kg/m², in CTRL, 5%. Only 1 patient in the T-group and 5 in CTRL had normal weight. In the T-group, weight decreased from 99.7±4.8 kg to 89.6±9.0 kg after 10 years (p<0.0001), waist circumference from 111.1±7.4 to 99.2±5.5 cm, BMI from 36.2±4.4 to 29.1±2.9 kg/m²; per cent weight loss was
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20.1±5.3% (p<0.0001 for all). In CTRL, these measures remained stable. In the T-group, all but 1 patient lost weight, despite the fact that 54 men were on insulin. The only patient who gained 1 kg of weight during the observation time was a man whose testosterone treatment was interrupted following a diagnosis of prostate cancer. In CTRL, most men gained weight. Only 12 men lost weight, and 5 men did not have any change in weight. In the T-group, 4 patients (3%) died of non-cardiovascular events. There were no non-fatal major adverse cardiovascular events (MACE). In CTRL, 24 patients (15.7%) died. 50 non-fatal MACE occurred (26 myocardial infarctions, 24 strokes).

Conclusion: Long-term treatment with TU in hypogonadal men with T2DM resulted in substantial and sustained weight loss in comparison to untreated controls. Mortality and MACE were reduced in the testosterone-treated group. Correcting hypogonadism in men with T2DM supports standard diabetes treatment including lifestyle instructions.

101 (Poster)
SEMIN QUALITY AND REPRODUCTIVE FUNCTION AS MARKERS OF GENERAL MALE HEALTH
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University of Padova
(Presented By: Alberto Ferlin, MD, PhD)

Introduction: Some evidence suggested that infertile men are at increased risk for hypogonadism, metabolic derangements and osteoporosis, have higher long-term morbidity and mortality than controls, but data are scarce and not conclusive. Objectives: Here we tested whether semen quality and reproductive function could represent a marker of general male health. Methods: This prospective cohort study included 5177 males of fertile couples. All subjects had semen analysis, reproductive hormones, testis ultrasound and biochemical determinations for glucose and lipid metabolism. Hypogonadism was defined as testosterone < 10.5 nmol/L and/or LH > 9.4 IU/L. Subjects with total sperm count (TSC) <10 million had genetic testing (karyotype, Y chromosome microdeletions, CFTR gene mutations) and those with hypogonadism dual-energy x-ray absorptiometry for bone mineral density (BMD).

Results: Results obtained Men with low sperm count (<39 million/ejaculate) are at high risk of hypogonadism (OR 12.2, 95% CI 10.2-14.6), have higher BMI, waist circumference, systolic pressure, LDL-cholesterol, triglycerides, and HOMA-index, and lower HDL-cholesterol, and higher prevalence of metabolic syndrome (OR 1.246, 95 CI 1.005-1.545). All data are worse in men with low testosterone and milder, but significant, in men with isolated elevated LH. Low TSC per sé is associated with poor metabolic parameter. Men with hypogonadism have lower BMD and 51% prevalence of osteoporosis/osteopenia.

Conclusion: This large study suggests that low sperm count is associated with poorer metabolic, cardiovascular and bone health. Hypogonadism is mainly involved in this association, but low sperm count in itself is a marker of general health. Infertile patients have the great opportunity to benefit from the identification of diagnostic and prognostic markers, comorbidities and risk factors.

102 (Poster)
DETERMINING FACTORS THAT AFFECT THE FEASIBILITY OF AT-HOME SEMEN COLLECTION FOR PERSONAL CRYOPRESERVATION: WHAT CAN WE PROMISE PATIENTS THAT PARTICIPATE IN THIS TYPE OF SPERM BANKING? A PILOT STUDY
Betsy Cairo PhD, Lauren Goedde, MS, and Juliana Tyo MS
CryoGam Colorado
(Presented By: Betsy Cairo, PhD, HCLD)

Introduction: Off-site semen collection for cryopreservation and storage would be beneficial for patients that do not have access to a semen processing and long-term storage laboratory. The premise of this pilot study was to ascertain what parameters could most affect the outcome of specimens collected off-site and shipped to our facility for processing. Objectives: The purpose of this pilot study is to determine the feasibility of a mail-in cryopreservation program with respect to survivability. Methods: Anonymous donors currently in CryoGam’s donation program were utilized as their own control due to historical data regarding the donors’ ejaculate parameters. Donors collected an ejaculate at home into a sterile specimen cup. Immediately after ejaculation, donors were instructed to pour 10mLs of Hams F-10 (in kit) into the specimen cup containing the ejaculate. The cup was placed into a small blood transport bag containing absorbent paper and then into a 7” x 9” PCM ambient shipper with a foam box insert. A pre-printed FedEx label was placed on the outside of the box and the package taken to a Fed Ex Drop-off location closest to their residence on the day the specimen was collected. Each donor submitted three samples. Processing of specimen began within 30 minutes of arrival via FedEx. Total transit time was calculated upon arrival. The following parameters were examined pre-freeze: 1.Volume 2. Count 3. Motility 4. Speed 5. Direction 6. Agglutination. Weather temperatures for the day the specimen was in transport were also documented. The specimen(s) were then processed according to a cryopreservation protocol. All specimens were pre-washed prior to freezing. A post-thaw evaluation was performed to determine survivability.

Results: A total of n=10 donors began the study with n=9 finishing as of to date. There were n=25 specimens evaluated. Average time from collection to delivery was 21.6 hours. Pre-process motility ranged from 40-80% (average 58%) and post thaw motility ranged from 1-60% (average 30%). There seemed to be a correlation between travel time and outside temperature with some donors but not with others. Note: Study is on-going.

Conclusion: Data suggests that home collection can be a viable option for distant populations that do not have access to care. Several parameters play an important role in predicting a positive outcome. However, due to the wide range of outcome care must be taken when counseling patients on potential outcome.

103 (Poster)
UTILITY OF AUTOLOGOUS SEMEN CRYOPRESERVATION SERVICES AT A SINGLE INFERTILITY CLINIC, A 10-YEAR REVIEW.
Karen Lockyear PhD¹, Sergey Moskovstein PhD, MD,³ Prati Sharma MD¹, Ari Baratz MD³, Karen Glass MD² and Clifford Librach MD³
¹CReATe Fertility Centre; ²CReATe Fertility Centre, University of Toronto; ³CReATe Fertility Centre, University of Toronto, Women’s College Hospital
(Presented By: Karen M. Lockyear, PhD)

Semen cryopreservation has become a routine practice in the treatment of infertility. Indications for banking sperm can include fertility preservation, banking of surgically retrieved spermatozoa, and increasingly, for fertility treatments; while surgically retrieved epidydimal or testicular sperm would be beneficial for patients that do not have access to a semen bank. Correcting hypogonadism in men with T2DM supports standard diabetes treatment including lifestyle instructions.
ABSTRACTS

represented 0.1%. Fertility preservation for oncology, degenerative diseases, transgender transitioning or prior to vasectomy represented 4.1%. The largest proportion (36.4%) of banked samples were utilized for fertility treatment, 4.3% were discontinued or transferred to other clinics, and 0.3% were used for testing purposes or donated to research. Of the patients utilizing their samples, 12.8% used all and 5.1% used more than half of their banked inventory. A large number of banked samples (59%) have not been utilized to date or have been abandoned and the patients unresponsive to all attempts at contact. This study provides data on the rates of banking and use of autologous semen samples at a single fertility clinic. Sperm freezing is increasingly incorporated as a part of fertility treatment, and there is great diversity in the type and purpose of banked samples. Therefore, decisions regarding semen cryopreservation must be made an individual basis; however, the practicality and carrying costs of processing and storage should be taken into account.

104 (Poster)
EFFECTS OF LONG TERM SHORT-ACTING TESTOSTERONE ADMINISTRATION BEFORE AND AFTER OOPHORECTOMY ON GONADOTROPIN SECRETION IN TRANS MEN
Luize Palaor MD¹, Carmen Alves MD¹, Vinicius Brito MD, PhD¹, Flavia Cunha MD, PhD¹, Berenice Mendonça MD, PhD, Prof¹, Jose Antonio Mancondes MD, PhD, Prof¹, Sorahia Domenice MD, PhD¹ and Elaine Costa MD, PhD, Prof²
¹Division of Clinical Endocrinology, Hospital das Clínicas, Department of Endocrinology of University of São Paulo School of Medicine, São Paulo, Brazil; ²Division of Clinical Endocrinology, Hospital das Clínicas, Department of Endocrinology of University of São Paulo School of Medicine, São Paulo, Brazil/ Androciense
(Presented By: Elaine F. Costa, MD, PhD, Prof)

Introduction: Testosterone is the key hormone in Trans men treatment. Regulation of gonadotropins secretion involves a complex balance between hypothalamic GnRH stimulation, inhibitory feedback by gonadal sex steroids, inhibin B and anti-mullerian hormone (AMH), and autocrine/paracrine modulation by hormones within the pituitary. However, the effects of long-term testosterone administration and gonadectomy on gonadotropin secretion in Trans men are poorly described. We aimed to report the effects of long-term short-acting testosterone administration before and after oophorectomy on gonadotropin secretion in Trans men. We conducted a retrospective analysis of 13 Trans men taking short-acting testosterone esters IM in a dose that ranged from 200-250 mg every two or three weeks for at least 3 years before and after oophorectomy and evaluated LH, FSH, T and E2 levels. Numerical variables were compared by one-way analysis of variance (ANOVA) followed by post-hoc Tukey’s test. Statistical significance was set at p<0.05.

Results: Our data showed that long-term testosterone treatment in Trans men resulted in significantly increase of serum T levels from 50.53 ± 28.46 ng/dl in the basal levels to 505.5 ± 161.7 ng/dl post 3 years treatment (p<0.001). LH levels decreased from 6.65 ± 4.52 U/L in basal state to 2.06 ± 2.13 U/L after testosterone treatment (p =0.51). FSH level decreased from 6.0 ± 2.66 in basal state to 5.52 ± 8.89 U/L after testosterone replacement (p=0.99). Interestingly, the mean serum LH and FSH levels after gonadectomy was significantly higher than before gonadectomy, even during testosterone treatment - 15.4 ± 15.1 U/L (p=0.014) and 32.8 ± 32.4 U/L (p=0.012), respectively. No statistical difference in T and E2 levels was found – mean of 439.7 ± 267.5 ng/dl (p=0.702) and 39.8 ± 37.1 ng/dl (p=0.28), respectively.

Discussion and Conclusion: Serum T levels after long term testosterone treatment were higher than prior to treatment and were maintained after gonadectomy. Therefore, the slight suppressive effects of high T levels on LH levels prior to gonadectomy were attributable to androgen action rather than to androgen-derived estrogen action, since the basal E2 levels were similar to the ones after testosterone treatment. We also demonstrated an increase in gonadotropin levels after gonadectomy, despite the maintenance of testosterone levels, suggesting the influence of another hormonal factors related to the ovaries on gonadotropins levels.

105 (Poster)
TESTICULAR SPERM RETRIEVAL OUTCOMES IN AZOOSPERMIC MEN WITH NON-MOSAIC KLINEFELTER’S SYNDROME
Ta-Yao Tai MD and Yung-Ming Lin PhD
National Cheng Kung University Hospital
(Presented By: Ta-Yao Tai, MD)

Objective: To report the effects of long-term short-acting testosterone administration and gonadectomy on gonadotropin secretion in Trans men. We conducted a retrospective analysis of 13 Trans men taking short-acting testosterone esters IM in a dose that ranged from 200-250 mg every two or three weeks for at least 3 years before and after oophorectomy and evaluated LH, FSH, T and E2 levels. Numerical variables were compared by one-way analysis of variance (ANOVA) followed by post-hoc Tukey’s test. Statistical significance was set at p<0.05.

Results: Increased serum FSH and LH levels as well as decreased testosterone levels were noted in the majority of the patients. All patients had bilateral testicular atrophy (less than 15 ml for each testicle). Five patients were between the age of 18 to 29 years at the time of operation, while the other 28 patients were age 30 years or older. Three out of five (60%) younger patients had successful TESE and nine out of 28 (32.1%) older patients met with success (P = 0.327). The overall successful sperm retrieval rate was 36.4% (12/33). For 12 patients with successful TESE, the histopathological findings were hypopsematogenesis in 8, late maturation arrest (MA) in 2, early MA 1 and Sertoli cell-only syndrome (SCOS) in 1. For 21 patients with failed TESE, there were hypospermogenesis in 1, early MA 1 and SCOS in 19.

Conclusion: TESE is an effective sperm retrieval technique in men with non-mosaic KS, and successful sperm retrieval might decrease with age.

106 (Poster)
SERUM LEPTIN LEVELS ARE STRONGLY ASSOCIATED WITH BODY FAT MASS BUT NOT WITH CARDIO-METABOLIC RISK FACTORS OR INSULIN RESISTANCE WITH ANDROGEN DEFICIENCY IN GEORGIAN STUDY
Shota Janjigava, PhD
National Institute of Endocrinology
(Presented By: Shota Janjigava, PhD)

Objective: Metabolic syndrome and obesity is a chronic disease that concerns over a billion people all over the world. Adipose tissue is a place of synthesis of several metabolically active proteins, called adipokines. One of such adipokines is – leptin. The aim of present study is to find correlation between leptin and risk factors of cardio-metabolic disease and androgen deficiency.

Materials and Methods: The case-control study was conducted in a group of Georgian people. A total of 186 participants aged 20-70 were included for the study. The subjects who were overweight or obese were enrolled in the study group, whereas the subjects with normal weight were enrolled in the control group. The control group consisted of 20 subject with normal weight. In both groups following measurements were done: assessment of height, weight, BMI, waist circumference, blood pressure. Venus blood sample was obtained for plasma leptin, insulin, glucose and lipid profile analysis. The risk of cardio-vascular disease was calculated according the Framingham heart risk calculator. Body fat distribution was measured using Dual energy X-ray Absorbiomter. Statistical analyses were performed using the SPSS 19.0 software package (SPSS, Inc., Chicago, IL).

Results: Our data showed that long-term testosterone treatment in Trans men resulted in significantly increase of serum T levels from 50.53 ± 28.46 ng/dl in the basal levels to 505.5 ± 161.7 ng/dl post 3 years treatment (p<0.001). LH levels decreased from 6.65 ± 4.52 U/L in basal state to 2.06 ± 2.13 U/L after testosterone treatment (p =0.51). FSH level decreased from 6.0 ± 2.66 in basal state to 5.52 ± 8.89 U/L after testosterone replacement (p=0.99). Interestingly, the mean serum LH and FSH levels after gonadectomy was significantly higher than before gonadectomy, even during testosterone treatment - 15.4 ± 15.1 U/L (p=0.014) and 32.8 ± 32.4 U/L (p=0.012), respectively. No statistical difference in T and E2 levels was found – mean of 439.7 ± 267.5 ng/dl (p=0.702) and 39.8 ± 37.1 ng/dl (p=0.28), respectively.

Discussion and Conclusion: Serum T levels after long term testosterone treatment were higher than prior to treatment and were maintained after gonadectomy. Therefore, the slight suppressive effects of high T levels on LH levels prior to gonadectomy were attributable to androgen action rather than to androgen-derived estrogen action, since the basal E2 levels were similar to the ones after testosterone treatment. We also demonstrated an increase in gonadotropin levels after gonadectomy, despite the maintenance of testosterone levels, suggesting the influence of another hormonal factors related to the ovaries on gonadotropins levels.
Introduction: Laboratory techniques were frequently develop to optimize sperm quality to Assisted Reproduction. Sperm incubation is a methodology applied in low quality sperm, aimed to promove in vitro sperm maturation and, consequently, to improve motility rate.

Objective: To evaluate sperm motility quality in asthenozoospermic samples during prolonged in vitro incubation under aerobic conditions.

Methods: Were used 12 asthenozoospermic (progressive motility <32%) seminal samples from infertile patients (21-45 years old), referred to an andrology laboratory. Sperm samples were classified as T0 (fresh samples), T1 (1 hour incubation), T2 (2 hours incubation), T3 (3 hours incubation) and T4 (4 hours incubation). After the collection by masturbation, seminal parameters were evaluated (T0, WHO; 1999, WHO; 2010), covered with mineral oil and incubated at 37ºC in a CO2 atmosphere (5%). For statistical analysis, we used analysis of variance Test (ANOVA) and Pearson correlation coefficient test.

Results: A significant reduction was observed in total motile sperm number (p=0.001) in samples incubated (T1, T2, T3 and T4; p<0.001) when compared with fresh samples. Incubation by 2 hours increased total motility when compared with fresh sample. In addition, incubation by 4 hours decreased the total motility (p<0.050). There was negative correlation between sperm incubation time and the parameters: total motile sperm number (r= - 0.362, p= 0.006)(Table 1).

Conclusion: Sperm incubation at aerobic environment by 2 hours showed positive benefits in sperm motility. This methodology can be applied in asthenozoospermic samples. On the other hand, semen samples should not be incubated to 4 hours or more because there are motility damage.

Financial Support: FAPESP (nº 2017/03599-1)/Androscience

Keywords: Incubation, sperm, motility, asthenozoospermia, sperm function.

Ethics Committee Approval: FMUSP Ethics Committee (2.086.727/2017)
DETERMINATION OF HABITS AND LIFESTYLES THAT AFFECTS SPERM MOTILITY QUALITY IN MEN

Dayane Reis BSc¹,², Juliana Pariz PhD³,⁴, Victoria Coutinho BSc student¹,³, Caroline Ranéa MSc student¹,³, Inari Ciccone MSc student¹,³, Rosa Alice Monteiro BSc¹ and Jorge Hallak MD, PhD¹,³
¹Androscience – Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Laboratory, Sao Paulo, Brazil; ²Methodist University of Sao Paulo, Brazil; ³Dept. of Urology, Universidade de São Paulo, Brazil; ⁴Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil

(Submitted By: Dayane Reis, BSc)

ABSTRACT

Introduction: Asthenozoospermia is a condition frequently associated with male infertility, characterized by reduction of progressive motility (<32%). However, the habits and lifestyles causing asthenozoospermia are still unclear.

Objective: To determine the habits and lifestyles that affects sperm motility quality.

Methods: In this retrospective study, was analyzed 659 medical records of patients between 18 and 45 years-old, between 2000 and 2017. Patients were classified into 4 groups: fertile normozoospermic (control group), fertile asthenozoospermic, infertile normozoospermic and infertile asthenozoospermic. Was evaluated semen and functional parameters and correlated with habits and lifestyles (overweight, sedentary lifestyle, more than 9 hours of daily work, water consumption, coffee consumption, alcoholic beverages consumption and soda consumption). Were calculated the frequencies and correlations by Sperm and Pearson test.

Results: The fertile asthenozoospermic and infertile asthenozoospermic groups presented, respectively, a high frequency in patients with overweight (57% and 75%), sedentarism (74.1% and 93.2%), more than 9 hours of work (89.5% e 100%), consumption of less than 1 liter of water (63% and 91.8%), coffee consumption (46.3% and 49.3%), consumption of alcoholic beverages (46.3% and 33.3% %) and soda consumption (34.8 and 38.7). Sedentarism demonstrated negative correlation with total (r=-0.161; p<0.001) and progressive motility (r=-0.170; p<0.001). Sedentarism demonstrated negative correlation with total (r=-0.161; p<0.001) and progressive motility (r=-0.170; p<0.001). Total (r=-0.131; p<0.001) and progressive (r=0.267; p<0.001) motility have positive correlation with coffee consumption.

Conclusion: We can suggest that sedentarism, more than 9 hours of daily work and soda consumption impairs seminal quality, reducing the progressive motility of the spermatozoa and contributing to the condition of asthenozoospermia. On the other hand, habits such as drinking water, coffee and alcoholic beverages favored increased sperm motility.

Financial Support: Androscience/FAPESP (n° 2016/15009-1)

Ethics Committee Approval: FMUSP Ethics Committee (n°859215/2014)

Keywords: Sperm motility, habits and lifestyle, male fertility, asthenozoospermia, semen, sperm.

DAILY INGESTION OF THE COMBINATION OF GINGER, MUIRA PUAMA, PAULLINA CUPANA AND L-CITRULLINE IMPROVES ERECTILE FUNCTION.

Sabine Nguyen DO¹, Magda Shaheen MD¹, Senait Teklehaianmat PhD², Dulcie Kermah PhD¹, Monica Ferrini MS, PhD¹ and Jacob Rajfer MD²
¹Charles R. Drew University; ²UCLA

(Presented By: Jacob Rajfer, MD)

Introduction and Objectives: Inducible nitric oxide synthase (iNOS) has been shown to be upregulated within the corporal smooth muscle cells (CSMC) in the rat as it ages. This upregulation is considered the result of an attempt by the CSMC to combat the normal aging-related CSMC apoptosis and corporal fibrosis that leads to erectile dysfunction (ED). In the rat, the nutraceutical combination of ginger, Muira puama, Paullinia cupana and L-citrulline (COMB-4) was reported to increase iNOS and cGMP in vitro and iNOS, guanylyl cyclase and cGMP in vivo in the CSMC. To determine whether such daily treatment of COMB-4 in men could translate into a demonstrable early improvement in erectile function, a 3-month pilot study was conducted in aged men.

Methods: Following IRB approval, adult men with ED who enlisted into the study consented to take COMB-4, the combination of ginger (500mg/day), muira puama (500mg/day), paullinia cupana (500mg/day) and L-citrulline (1600mg/day), twice daily for three months. They were requested to abstain from all ED therapies during the study. Participants completed the International Index of Erectile Function (IIEF-15) survey during their initial (B), first (M1), second (M2), and third (M3) month clinical visits. To evaluate for change in ED with COMB-4 treatment, scores from questions 3 (Q3) and 4 (Q4) of the erectile function domain, which corresponds to the ability to achieve and maintain an erection, were analyzed over time. Wilcoxon signed-rank test for paired data was used. P<0.05 was considered statistically significant.

Results: Fifty-three adult males with mean age of 57.8 ± 10.7 years initially participated in the trial, with 16 patients remaining by M3. A statistically significant increase in Q3 score was seen at M1 and M2 compared to baseline. Q3 median scores were: B=3, M1=4, M2=4, M3=4. For Q4, a statistically significant increase in score was seen at M1, M2, and M3 compared to baseline. Q4 median scores were: B=3, M1=3, M2=4, M3=3.

Conclusion: In this study, an early improvement in erectile function can be seen in aged men who took COMB-4 daily. This clinical observation may be attributed to the known stimulation by COMB-4 of the NO-cGMP pathway via iNOS within the CSMC. Further investigation may be required to evaluate the longer-term treatment with this nutraceutical combination.

VASECTOMY REVERSAL UTILIZING FIBRIN GLUE REINFORCEMENT: ONE INSTITUTION’S EXPERIENCE

Graham Machen MD, Colin Kleinguetl MD, Wencong Chen PhD and Erin Bird MD, MBA
Scott and White Medical Center

(Presented By: Graham Luke Machen, MD)

More than 500,000 men in the United States have a vasectomy annually. Around 2-6% of these individuals later elect to have a vasectomy reversal. Vasovasostomies (VVs) are typically performed using a single or double layer closure, with no difference having been demonstrated between the two in terms of success rates. In 2005, Ho et al described a microscopic technique in which 3 full-thickness sutures are used and the anastomosis is reinforced with fibrin glue. At our institution, a similar technique has been used for greater than 10 years. We set out to describe our experience and results.

A retrospective chart review was undertaken from Jan 1 2007 to Dec 31 2016. Patient demographic information, operative data, and post op results were analyzed. For the purpose of this study, vasoepididymostomies were excluded. In every case, a modified single layer anastomosis was performed using 9-0 full thickness sutures. The anastomosis was then...
reinforced with fibrin glue. Patency was defined as semen present in post operative analysis and/or pregnancy, and rates were analyzed using a multiple logistic regression adjusting for age, OR time, and BMI. See Table 1 for summary of results. Over a 10 year period, 314 VVs were performed. An average of 6 sutures were used per side. Patency data was available on 150 of these patients. The overall patency rate was 88.7%, and rates comparing individuals who had their vas reversal within 10 years of vasectomy reached statistical significance with an odds ratio of 3.71; p=0.041.

In 2005, Ko et al demonstrated that a 3-suture VV using fibrin glue may be a feasible technique, especially with vas intervals 8 years or less. Our study demonstrates that by using approximately 6 sutures with a reinforcement layer of fibrin glue, patency rates remain around 90% out to 14 years s/p vasectomy. Additionally, our results demonstrate a higher likelihood of success if the vas interval was 10 years or less. Further, with a mean OR time of 95 minutes, this technique would likely provide cost savings, as most other reports typically describe operative times ranging from 120-165 minutes. Thus, fibrin glue assisted VV seems to be reasonable in men who have had their vasectomy within 14 years.

Table 1. Summary of Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (0.92, 1.13)</td>
<td>0.709</td>
</tr>
<tr>
<td>OR time</td>
<td>1.00 (0.97, 1.03)</td>
<td>0.852</td>
</tr>
<tr>
<td>BMI</td>
<td>1.07 (0.95, 1.20)</td>
<td>0.265</td>
</tr>
<tr>
<td>Interval after Vasectomy (years)</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>≤10 years</td>
<td>3.71 (1.05, 12.05)</td>
<td></td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

I12 (Poster)
PREPARATION TECHNIQUES INFLUENCE HUMAN SPERM KINEMATICS AND SEX CHROMOSOME RATIO
Ibukun Oyeyipo BSc, MSc, PhD, Michelle van der Linde BSc, MSc and Stefan du Plessis BSc, MSc, PhD, MBA
Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa.
(Presented By: Ibukun Oyeyipo, BSc, MSc, PhD)

Introduction: Currently, routine semen preparation techniques do not specifically target or consider inherent sperm characteristics that may impact on separating for X and Y chromosome bearing spermatozoa. Aims: To investigate the influence of different preparation techniques on the ratio of X and Y chromosome bearing spermatozoa.

Methods: 27 semen samples obtained from healthy volunteers (19-26 years old) were subjected to four preparation techniques: direct swim-up, capillary tube migration, double density gradient centrifugation and double wash. Assessment of the effects of each technique on the ratio of X and Y chromosome bearing spermatozoa was carried out. Changes in the sex-chromosome ratio of samples were established by double-labeled fluorescent in situ hybridization, while sperm motility and velocity parameters were assessed using computer-aided sperm analysis.

Results: There was successful enrichment of Y-chromosome bearing spermatozoa through a direct swim-up method as well as discontinuous gradient centrifugation. After direct swim up procedure, the percentage of total motility increased significantly between the neat sample and fraction A (67.75 ± 4.774% vs. 97.70 ± 4.420%, p=0.0007) as well as progressive motility between the two fractions (39.72 ± 6.270% vs. 83.53 ± 6.270%, p=0.0001). A significant increase in the curvilinear velocity (VCL) and straight line velocity (VSL) between the control and Fraction A (VCL: 46.42 ± 6.273µm/s vs. 78.84 ± 5.834µm/s, p=0.002; VSL: 16.00 ± 2.792µm/s vs. 26.86 ± 2.707µm/s, p=0.0003) was also observed.

Conclusion: This study demonstrated the potential role of different techniques in influencing the distribution of X and Y chromosome bearing spermatozoa. Hence, it is an important factor to put into consideration during assisted reproductive techniques.

Financial Support: Harry Crossley Foundation (South Africa)
Keywords: Sperm preparation techniques, Sex-chromosomes, Motility, Velocity, FISH.

Ethics Committee Approval: S13/04/068

113 (Poster)
EVALUATION OF SERIAL THAWING-REFREEZING ON HUMAN SPERMATOZOA RESISTANCE: MITOCHONDRIAL AND DNA INTEGRITY PERSPECTIVE
Rosa Alice Monteiro BSc¹, Juliana Pariz PhD²,³,⁴, Bruna Zillig BSc student²,²,²,²,², Elena Faquinetti BSc student²,², Caroline Ranea MSc student²,²,²,², Donald Evenson PhD² and Jorge Hallak MD, PhD²,³,⁴,⁵
¹Androscience – Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Laboratory, Sao Paulo, Brazil; ²University of Santo Amaro, Sao Paulo, Brazil; ³Methodist University of Sao Paulo, Brazil; ⁴Dept. of Urology, Universidade de Sao Paulo, Brazil; ⁵Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil; ⁶SCSA Diagnostics
(Presented By: Rosa A. Monteiro BSc)

Introduction: Sperm cryopreservation is an important tool for the preservation of gametes, provide a chance to establish pregnancy by Assisted Reproduction Techniques (ART). Sample thaw to ART procedures requires some spermatozoa in each cycle, and the remaining sperm are discarded, since it is believed that the sample becomes nonviable to refreeze. Objective: To evaluate the resistance rate (motility, cryosurvival, DNA integrity and mitochondrial activity) of human spermatozoa after three refreeze-thaw cycles.

Methods: Were used 22 cryopreserved semen samples with total motility above 52% and cryosurvival rate ≥22%. Fresh samples were cryopreserved with Test Yolk Buffer in liquid nitrogen by slow freezing. Each sample was submitted to three serial process of thawing-refreezing. Was evaluated in each step: seminal parameters (World Health Organization, 2010), cryosurvival rate ([total motile sperm post-thaw]x100/[total motile sperm/ tube]), mitochondrial activity (3,3'-diaminobenzidine method) and DNA fragmentation index (DFI; SCSA method). Groups were compared by paired Student’s T and adopted p<0.05.

Results: Post-thawed results are presented in table 1. Sperm motility, cryosurvival rates and mitochondrial activity decreases significantly when thawing-refreezing two or three times. No difference was observed in DFI between groups. Conclusion: In this study, several injury by thawing-refreezing was observed, because spermatozoa motility do not resisted to repeated cryopreservation. A possible mechanism is mitochondrial injury, decreasing consequently sperm motility and cryosurvival rates. Therefore, sperm cryopreservation of a large number of samples/ aliquots is important to improve the patient success chances in ART cycles.

Financial Support: Androscience Ethics Committee Approval: FMUSP Ethics Committee
Keywords: Sperm, cryopreservation, DNA fragmentation index, mitochondrial activity, cryosurvival rates.

Table 1. Mean and standard deviation of sperm parameters after first, second and third post-refreeze samples evaluation

<table>
<thead>
<tr>
<th>Variable</th>
<th>First thaw (FT)</th>
<th>Second thaw (ST)</th>
<th>Third thaw (TT)</th>
<th>FT x ST</th>
<th>TT x ST</th>
<th>FT x TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total motile sperm number (10^6)</td>
<td>24.1±17.85</td>
<td>6.1±2.20</td>
<td>3.2±0.45</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>3.108</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>71.2±14.27</td>
<td>25.4±2.80</td>
<td>50±15.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Cryosurvival rate (%)</td>
<td>22.6±10.32</td>
<td>11.0±1.89</td>
<td>62.8±4.22</td>
<td>0.002</td>
<td>0.009</td>
<td>0.001</td>
</tr>
<tr>
<td>DNA fragmentation index (%)</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitochondrial activity (%)</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NE: Not evaluated
Parent Studied x T test: p<0.05

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CASE REPORT: VARICOCELECTOMY IMPROVES SEMEN QUALITY AND THE ABILITY OF SPERM TO UNDERGO CAPACITATION.

Eric Seaman MD¹ and Samuel Aly MD²
¹New Jersey Urology; ²Rutgers - New Jersey Medical School
(Presented By: Eric K. Seaman, MD)

Introduction: About 30% of infertile men have a clinically significant varicocele. Varicoceles may affect testicular function with respect to fertility as well as testosterone production. The utility of semen analysis measures to assess male fertility before and after varicocelectomy can be problematic, as their reference ranges are based on fertile men and can overlap significantly with observations obtained from those who are infertile. Cap-Score™ is a validated test of male fertility that uses GM1 localization patterns to measure capacitation ability and reflects male fecundity. In this case report, Cap-Score and semen quality measures were used to evaluate the success of varicocelectomy.

Methods: A 37-year-old Asian presented with secondary infertility after 12 months of trying to conceive. There was no change in his medical history since his last paternity. Evaluation included scrotal ultrasound. In addition, serum testosterone (T), the Cap-Score Sperm Function Test and the following standard semen analysis parameters were determined before and after surgery: semen volume, sperm density, percent motility and Kruger strict morphology.

Results: Scrotal sonography showed that the right and left testis had volumes of 10 and 11 cc.s. It also revealed varicoceles of 0.32 and 0.23 cm in the left and right pampiniform venous plexus. Pre- and Post-Operative values for T, Cap-Score and semen quality measures are shown in Table 1, with normal values and measurement scales shown in parentheses. Following varicocele correction, there was improvement in Cap-Score from abnormal to normal, as well as in semen parameters of percent motility and Kruger strict morphology.

Conclusion: Varicocelectomy positively impacted semen quality and a novel measure of sperm function, Cap-Score, which measures the ability of sperm to capacitate, a requisite for fertilization. Addition of Cap-Score to semen analysis may offer urologists a complementary tool to better evaluate the treatment of a host of causes of male infertility, including varicocele.

Table 1. Pre- and post-operative results

<table>
<thead>
<tr>
<th>Pre-Op</th>
<th>Post-Op</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (ng/dl)</td>
<td>Cap-Score</td>
</tr>
<tr>
<td>426</td>
<td>19.1</td>
</tr>
<tr>
<td>426</td>
<td>35.0</td>
</tr>
</tbody>
</table>

FOLLOW UP OF THE SURGICAL TREATMENT OF PENILE CURVATURE WITH 16-DOT PLICATION

Jianhua Guo
(Presented By: Jianhua Guo)

Objective: The aim is to assess the long-term satisfaction and functional results of surgical correction patients with penile curvature by 16-dot minimal tension plication.

Material and Methods: We retrospectively reviewed 56 cases of congenital penile curvature and 36 cases of Peyronie’s disease with penile curvature that underwent surgical correction by 16-dot minimal tension plication from August 2004 to December 2010 in our center. Patients were recommended daily low dose phosphodiesterase type 5 (PDE5) inhibitors two weeks after surgery and maintained for two months. At follow-up, recurrence rate, complications, erectile function, satisfaction with the surgery was assessed.

Results: Six patients were unable to contact. During the follow-up period (median time 79 months; range 35-107 months), there were no surgery-related complications, and 82 patients (95.3%) and 77 partners (89.5%) reported overall satisfaction after the surgery. Postoperative penile length shortening (median penile length loss 1.3 cm) was noted in 35 patients (40.6%), which did not affect their sexual intercourse. Complete penile straightening was achieved in 79 patients (91.8%). Slight residual curvatures were observed in 7 patients, and did not affect their sexual intercourse. In addition, no de novo erectile dysfunction was observed, and sexual function was significantly improved, especially in the patients who have received PDE5 inhibitors for two months after two weeks surgery.

Conclusion: Correction of penile curvature using 16-dot plication is a simple and safe method to achieve cosmetic and functional satisfaction in carefully selected patients.

YOGA AND MEDITATION: IMPACT ON DYSREGULATED SPERM TRANSCRIPTS IN RECURRENT PREGNANCY LOSS

Vidhu Dhawan, MD¹, Manoj Kumar MD², Dipika DeK MD³, Neena Malhotra MD¹, Neeta Singh MD¹, Vatsala Dadiwal MD³ and Rima Dada MD, PhD³
¹Laboratory for Molecular Reproduction & Genetics, Dept. of Anatomy, AIIMS; ²Dept. of Obstetrics & Gynaecology, AIIMS
(Presented By: Vidhu Dhawan, MBBS, MD)

Introduction: With an overgrowing spectrum of accepted etiopathologies for recurrent pregnancy loss (RPL) and evidences on abnormal embryo quality, the current paradigm has shifted towards the assessment and management of paternal contributions in early embryonic development. Apart from deranged oxidative stress parameters, the dysregulation in spermatozoal transcripts contributes significantly to RPL. Recent studies are highlighting the impact of yoga based lifestyle interventions (YBILI) in the restoration of seminal ROS levels and sperm DNA integrity. However its impact on sperm transcripts has not been evaluated.

Objectives: The present study was designed with an aim to evaluate the effect of brief YBILI on the expression pattern of spermatozoal FOXG1B, SOX3, OGG1, PARP1, RPS6, RBM9, RPS17 and RPL29 in male partners of couples experiencing RPL. Seminal oxidative stress (OS) and DNA Fragmentation Index (DFI) was also assessed.

Methods: The present study is a part of a prospective ongoing exploratory study and 30 male partners of couples with RPL were recruited from August 2016 to June 2017 in YBILI program. Semen samples were obtained both at baseline and at the end of active intervention (21 days) and also from 30 fertile controls but they were not enrolled in YBILI. Gene expression analysis was done by q-PCR and relative quantification of target genes was calculated after normalization to β-actin and GAPDH with 2-ddCt method. The levels of seminal OS and sperm DNA damage was assessed by measuring levels of reactive oxygen species (ROS) by chemiluminiscence and DFI by sperm chromatin structure assay (SCSA).

Results: The relative gene expression of the target genes was found to normalize towards that of control values at the end of active YBILI. The spermatozoal OGG1 and PARP1 were seen to be upregulated, while FOXG1B, SOX3, RBM9, RPS17 and RPL29 showed downregulation. A significant reduction in ROS levels, increase in motility, sperm count (done twice) and an insignificant decrease in DFI was also witnessed.

Conclusion: The adoption of YBILI as an adjunct to the management of RPL may not only help in reducing oxidative DNA damage but also in normalization of sperm transcript levels contributing to early embryogenesis. This may not only help in improving pregnancy outcomes but also improve the health trajectory of the offspring, and thus highlighting the therapeutic role of YBILI.

Key words: dysregulation, oxidative stress, sperm transcript, yoga, meditation.
ABSTRACTS

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118 (Poster)
MALE CONTRACEPTION INITIATIVE STRATEGY, RESEARCH, AND GRANT ACTIVITIES, 2017-18
Logan Nickels, Mitch Eddy PhD and David Sokal MD
Male Contraception Initiative
(Presented By: Logan Nickels, PhD)

Introduction: Roughly half of all pregnancies are unplanned. This creates a detrimental impact at both the societal and individual level. This persistent occurrence of unplanned pregnancies indicates that contraceptive needs are still not being met. The largest gap for contraceptive need is in men’s options. Men have only two modern contraceptive options available, and no reversible method that is as reliable as The Pill. Further, they have no long-acting reversible contraceptives.

Objective: Our objective is to promote the development of novel, reversible male contraceptives. In 2014, we founded a nonprofit organization to pursue this objective: the Male Contraception Initiative (MCI), a 501(c)3 nonprofit, at MaleContraceptive.org.

Methods: MCI is using multiple approaches: Promoting awareness, educating the public through media and educational resources. Performing research to answer questions regarding the potential impact and marketing of novel reversible male contraceptives. Informing policy, by networking with governmental and non-governmental agencies. Networking with and funding researchers, inside and outside the US, to help them advance their research, and providing connections and access to appropriate experts. Strategizing on the best approaches to develop and fund new, reversible male contraceptives.

Results: We have published research that estimates the potential impact of (a) a male Pill, and (b) a long-acting reversible contraceptive for men (e.g. reversible vasectomy), on unintended pregnancies in three countries. We have conducted an acceptability survey among men in the US, and are currently analyzing the results. We have focused initially on an R&D strategy to target sperm function and sperm transport rather than on methods that disrupt spermatogenesis. In early 2017 we issued a request for proposals and recently awarded a three-year, $500,000 grant to Vibliome Therapeutics to extend their work on a novel kinase inhibitor. We have completed the review of proposals for two-year, $150,000 “Seed Grants,” which are designed to promote studies to identify novel male contraceptives. Recipients of these awards were announced recently.

Conclusion: We are making significant progress in the promotion and support of male contraceptives, but success is many years away. We encourage you to participate in this important effort in any way you can.
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