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American Society of Andrology Spring Conference
7–10 May 2022

The official journal of the American Society of Andrology and the European Academy of Andrology

WILEY

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ABSTRACT

ASA abstracts 2022

SCHEDULE AT A GLANCE

The American Society of Andrology 47th Annual Conference
"The Journey of Male Fertility: From Embryo to Adult and Back Again"
May 7 - 10, 2022 | Estancia La Jolla Resort & Spa | La Jolla, CA
Program Chairs: James Hotaling, MD, MS, FECSM & Jon Oatley, PhD
Immediate Past President: Michael A. Palladino, PhD
All sessions will be located in the ***La Jolla Ballroom*** unless otherwise noted. Speakers and times are subject to change.

FRIDAY, MAY 06, 2022

2:00 p.m. - 6:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

SATURDAY, MAY 07, 2022

7:00 a.m. - 7:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

4:00 p.m. - 9:00 p.m. **Exhibits Open**
Location: La Jolla Ballroom Foyer

8:30 a.m. - 5:00 p.m. **ASA Andrology Lab Workshop***
"The Emerging Technologies in Laboratory Testing for Male Reproduction"
Location: Learning Theater

6:00 p.m. - 6:20 p.m. **President's Welcome and Opening Remarks**
Maria Christina W. Avellar, PhD
ASA President
Michael A. Palladino, PhD
ASA Immediate Past President

6:20 p.m. - 7:30 p.m. **EMIL STEINBERGER MEMORIAL LECTURE: Using Single Cell Genomics to Explore Testis Development, Spermatogonia and Spermatogenesis**
Supported by the Emil Steinberger Endowment Fund
Introducer: Michael A. Palladino, PhD
Bloomfield College
Speaker: Brad Cairns, PhD
University of Utah School of Medicine

7:30 p.m. - 9:00 p.m. **ASA Welcome Reception**
Location: La Jolla Ballroom Foyer

SUNDAY, MAY 08, 2022

6:30 a.m. - 8:00 a.m. **Past Presidents' Breakfast**
Location: Garden Suite

6:30 a.m. - 6:30 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 4:00 p.m. **Exhibits Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 8:00 a.m. **Continental Breakfast**
Location: La Jolla Ballroom Foyer

8:00 a.m. - 8:15 a.m. **ASA Distinguished Andrologist Award***
Supported by the Eugenia Rosemberg Endowment Fund
**Not CME Accredited*
Introducer: Gail Cornwall, PhD
Texas Tech University Health Sciences Center
Recipient: Jacquetta Trasler, MD, PhD
McGill University Health Centre

8:15 a.m. - 8:30 a.m. **ASA Distinguished Service Award***
Supported by the ASA Past Presidents Endowment Fund
**Not CME Accredited*
Introducer: Michael A. Palladino, PhD
Bloomfield College
Recipient: George Gerton, PhD
Perlman School of Medicine at the University of Pennsylvania

8:30 a.m. - 8:45 a.m. **ASA Impact Award***
Supported by the Eugenia Rosemberg Endowment Fund
Introducer: Susan Rothmann, PhD, HCLD
Fertility Solutions, Inc.
Recipient: Steven Schrader, PhD
Cincinnati, OH

8:45 a.m. - 9:45 a.m. **DIVERSITY LECTURE: Increasing Diversity in the Biomedical Workforce: What, Why and How**
Supported by the ASA Education Endowment Fund
Introducer: Peter Y. Liu, MBBS (Hons I), PhD, FRACP
The Lundquist Institute at Harbor-UCLA Medical Center
Speaker: Keith Norris, MD, PhD
University of California, Los Angeles

9:45 a.m. - 11:00 a.m. **Poster Session I**
**Not CME Accredited*
Location: Pacifica Ballroom

11:00 a.m. - 12:45 p.m. **SYMPOSIUM I: The Aging Male Gamete: Implications for Population Health**
Moderators: Tessa Lord, PhD
University of Newcastle
Mary Samplaski, MD
University of Southern California

11:00 a.m. - 11:25 a.m. **Paternal Age Effect Mutations in the Male Germline**
Anne Goriely, PhD
University of Oxford

11:25 a.m. - 11:50 a.m. **How can we Select Optimal Gametes from Older Fathers?**
Jim Hotaling, MD
University of Utah School of Medicine

11:50 a.m. - 12:15 p.m. **Paternal Age and Offspring Somatic Health**
Michael Eisenberg, MD
Stanford University School of Medicine

12:15 p.m. - 12:30 p.m. **Q&A**

12:30 p.m. - 12:45 p.m. **Oral Abstract Presentations**

12:45 p.m. - 2:15 p.m. **Industry Sponsored Product Theater**

12:30 p.m. - 2:00 p.m. **MENTORING LUNCHEON: Navigating Career Paths in Andrology**
Sponsored by the Diversity and Trainee Affairs Committees
**Not CME Accredited*
Location: Grande Room
Academic Perspective: Ricardo P. Bertolla, DVM, PhD
HCLD Perspective: Dolores Lamb, PhD
Non-Academic/Industry Perspective: G. Charles Ostermeier, PhD

12:45 p.m. - 2:15 p.m. **Editorial Board Luncheon**

2:15 p.m. - 4:40 p.m. **SYMPOSIUM II: Sperm & Embryonic Competence and the Commitment to Meiosis**
Moderators: John McCarrey, MS, PhD
Univeristy of Texas at San Antonio
Jacquetta Trasler, MD, PhD
McGill University Health Centre

2:15 p.m. - 2:40 p.m. **Epigenetic Programming and the 3D Genome Organization in Male Germ Cells**
Satoshi Namekawa, PhD
Univeristy of California, Davis

2:40 p.m. - 3:05 p.m. **DNA Methylation in Gametogenesis and Embryogenesis**
Deborah Bourchis, PhD
Curie Institute

3:05 p.m. - 3:30 p.m. **Regulation of Meiotic Entry and Progression**
Devanshi Jain, PhD
Rutgers University

3:30 p.m. - 3:55 p.m. **Regulation of Meiotic Recombination**
Paula Cohen, PhD
Cornell University

3:55 p.m. - 4:25 p.m. **Q&A**

4:25 p.m. - 4:40 p.m. **Oral Abstract Presentations**

4:40 p.m. - 5:00 p.m. **Break**

5:00 p.m. - 6:00 p.m. **EUROPEAN ACADEMY OF ANDROLOGY LECTURE: Sperm Chromatin and Proteomic Contribution to the Zygote**
Introducer: Patricia Cuasnicu, PhD
Instituto de Biologia y Medicina Experimental
Speaker: Rafael Oliva, PhD
University of Barcelona

6:00 p.m. - 7:30 p.m. **SYMPOSIUM III: Balancing Transparency and Privacy of Donor Gametes and Offspring**
**Organized by the Trainee Affairs Committee and supported by the CREATE Endowment Fund*
Debate Panelists:
Moderator: Darya Tourzani
Transparency: Lisa Campo-Engelstein, PhD
University of Texas Medical Branch
Privacy: Vincent Couture, PhD
Laval University
Industry: Molly O'Brien, Esq
International Fertility Law Group

8:15 p.m. - 9:15 p.m. **Trainee Mixer**
Sponsored by Program Committee & Trainee Affairs Committee
Location: La Jolla Ballroom

MONDAY, MAY 09, 2022

6:30 a.m. - 6:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 3:30 p.m. **Exhibits Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 8:00 a.m. **Continental Breakfast**
Location: La Jolla Ballroom

8:00 a.m. - 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE: Return of a Forgotten Hero - *Zfy* Roles in Male Reproduction**
Supported by the Women in Andrology Endowment Fund
Introducer: Celia Santi, MD, PhD (WIA Chair)
Washington University School of Medicine
Speaker: Monika Ward, PhD
Institute for Biogenesis Research

9:00 a.m. - 9:15 a.m. **Break**

9:15 a.m. - 9:30 a.m. **Matthew P. Hardy Young Andrologist Award***
Supported by the Matthew P. Hardy Endowment Fund
**Not CME Accredited*
Introducer: John McCarrey, MS, PhD
University of Texas, San Antonio
Recipient: Brian Hermann, PhD
University of Texas, San Antonio

9:30 a.m. - 9:40 a.m. **Outstanding Trainee Investigator and Trainee Awards**
Supported by the CREATE Fund
**Not CME Accredited*

9:40 a.m. - 10:40 a.m. **INTERNATIONAL LECTURE: New Insights on Male Precocious Puberty**
Supported by the ASA General Endowment Fund
Introducer: Patricia Cuasnicu, PhD
Instituto de Biologia y Medicina Experimental
Speaker: Ana Claudia Latronico, MD, PhD
Universidade de Sao Paulo

10:40 a.m. - 12:00 p.m. **Poster Session II**
**Not CME Accredited*
Location: Pacifica Ballroom

12:00 p.m. - 1:30 p.m. **Lunch on Own**

12:00 p.m. - 1:30 p.m. **Women in Andrology Luncheon**
Sponsored by the Women in Andrology Committee
**Not CME Accredited*
Location: Grande Room
Host: Celia Santi, PhD
Washington University School of Medicine

1:30 p.m. - 3:00 p.m. **SYMPOSIUM IV: Embryonic and Fetal Origins of the Male Germline**
Moderators: Miles Wilkinson, PhD
University of California, San Diego
Brian Hermann, PhD
University of Texas, San Antonio

1:30 p.m. - 1:55 p.m. **Embryonic Origins of the Germline**
Amander Clark, PhD
Universtiy of California, Los Angeles

1:55 p.m. - 2:20 p.m. **Androgen Receptor Signaling and Endocrine Disruption in the Fetal Prospermatogonial Development**
Diana Laird, PhD
Universtiy of California, San Francisco

2:20 p.m. - 2:45 p.m. **Testis Formation in the Human Fetus**
Jingtao Guo, PhD
Universtiy of Utah School of Medicine

2:45 p.m. - 3:00 p.m. **Q&A**

3:00 p.m. - 3:20 p.m. **Break**

3:20 p.m. - 5:00 p.m. **SYMPOSIUM V: The Intersection of Metabolism and Male Reproductive Health**
Moderators: Michael Eisenberg, MD
Stanford University School of Medicine
Tracy Clement, PhD
Texas A&M University

3:20 p.m. - 3:45 p.m. **Leptin Signaling in Male Reproduction**
Gwen Childs, PhD
Universtiy of Arkansas Medical Sciences

3:45 p.m. - 4:10 p.m. **Modulation of Spermatogonial Stem Cell Activity by Hypoxia and Hypoxia-Driven Metabolic Pathways**
Tessa Lord, PhD
University of Newcastle, Australia

4:10 p.m. - 4:35 p.m. **Metabolite Control Over Mouse Sperm Motility**
Chris Geyer, PhD
East Carolina University

4:35 p.m. - 4:50 p.m. **Q&A**

4:50 p.m. - 5:00 p.m. **Oral Abstract Presentations**

5:00 p.m. - 5:30 p.m. **National Institute of Health Updates**

5:00 p.m. - 5:15 p.m. **NICHD Update**
Travis Kent
National Institutes of Child Health and Human Development

5:00 p.m. - 5:15 p.m. **NIEHS Update**
Thaddeus Schug, PhD
National Institutes of Environmental Health Sciences

5:30 p.m. - 6:30 p.m. **ASA Annual Business Meeting**

7:00 p.m. - 12:00 a.m. **ASA Annual Banquet**

TUESDAY, MAY 10, 2022

7:00 a.m. - 8:00 a.m. **2023 Program Committee Meeting**
Location: Dracena Room

7:00 a.m. - 12:30 a.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 8:00 a.m. **Continental Breakfast**
Location: La Jolla Ballroom Foyer

8:00 a.m. - 9:00 a.m. **AUA LECTURE: The Future of Andrology**
Sponsored by the AUA Educational Grant
Introducer: Jim Hotaling, MD
University of Utah School of Medicine
Speaker: Craig Niederberger, MD
University of Illinois at Chicago

9:00 a.m. - 9:15 a.m. **Poster Awards**
**Not CME Accredited*

9:15 a.m. - 9:30 a.m. **Break**

9:30 a.m. - 10:30 a.m. **AMELAR LECTURE: Are Sperm Counts Declining Worldwide?**
Supported by the Richard D. Amelar Endowment Fund
Introducer: Susan Rothmann, PhD, HCLD
Fertility Solutions, Inc.
Speaker: Shanna Swan, PhD
University of Rochester

10:30 a.m. - 12:00 p.m. **SYMPOSIUM VI: Debate: Hormonal vs Non Hormonal Approaches to Male Contraception**
Moderators: John Amory, MD, MPH, MSc
University of Washington
Wipawee "Joy" Winuthayanon, BSN, PhD
Washington State University

10:30 a.m. - 11:00 a.m. **Non-Hormonal**
Wei Yan, MD, PhD
The Lundquist Institute at Harbor-UCLA

11:00 a.m. - 11:30 a.m. **Hormonal**
Stephanie Page, MD, PhD
University of Washington

11:30 a.m. - 11:35 a.m. **Rebuttal - Non-Hormonal**

11:35 a.m. - 11:40 a.m. **Rebuttal - Hormonal**

11:40 a.m. - 11:50 a.m. **Q&A**

11:50 a.m. - 12:00 p.m. **Oral Abstract Presentations**

12:00 p.m. - 12:15 p.m. **Closing Remarks**

MESSAGE FROM THE IMMEDIATE PAST PRESIDENT

Michael A. Palladino, PhD
Immediate Past President

Dear Colleagues,

On behalf of the American Society of Andrology, I welcome you to the 47th Annual Conference at the Estancia La Jolla Resort & Spa, La Jolla, California, May 7-10, 2022! The theme of our program is "The Journey of Male Fertility: From Embryo to Adult and Back Again." I greatly appreciate the excellent work of Program Committee co-chairs Dr. Jon Oatley and Dr. James Hotaling for leading program development, and to all the Committee members involved in helping us plan our conference. Our 47th gathering is our first in-person conference since 2019, and is preceded by the XXVIth North American Testis Workshop, May 4-7, 2022.

Despite all of the travel and logistical challenges for conference attendees and organizers alike, our members have expressed great excitement to rejoin colleagues in person for the collaborative and networking benefits that are the essence of the ASA. In this regard, we all owe a special thanks to everyone our management team at Veritas Association Management for working tirelessly to ensure a successful and safe meeting.

On Saturday, May 7th, we offer the Andrology Lab Workshop: "The Emerging Technologies in Laboratory Testing for Male Reproduction." Opening remarks and our opening plenary for the Annual Conference program begin Saturday evening with the Emil Steinberger Memorial Lecture, "Using Single Cell Genomics to Explore Testis Development, Spermatogonia and Spermatogenesis," presented by Brad Cairns, PhD, from the University of Utah School of Medicine. As always, the reception that follows is a great chance to reconnect with our colleagues and to meet new colleagues.

Six symposia showcase the diversity of andrology and important science from the embryo through aging males:

- The Aging Male Gamete: Implications for Population Health
- Sperm and Embryonic Competence and Commitment to Meiosis
- Balancing Transparency and Privacy of Donor Gametes and Offspring, organized by the Trainee Affairs Committee
- Embryonic and Fetal Origins of the Male Germline
- The Intersection of Metabolism and Male Reproductive Health
- Debate: Hormonal vs. Non-Hormonal Approaches to Male Contraception

Each symposium highlights exciting work in basic science, translational medicine, and clinical practice, and demonstrates the value of collaboration between the bench and bedside with lectures presented by international experts and thought leaders in andrology. Important lectures you will not want to miss include:

- AUA Lecture: The Future of Andrology by Craig Niederberger, MD.
- Diversity Lecture: Increasing Diversity in the Biomedical Workforce: What, Why, and How by Keith Norris, MD, PhD.
- EAA Lecture: Sperm Chromatin and Proteomic Contribution to the Zygote by Rafael Oliva, PhD.
- International Lecture: New Insights on Male Precocious Puberty by Ana Claudia Latronico, MD, PhD.
- Women in Andrology Lecture: Return of a Forgotten Hero – Zfy Roles in Male Reproduction by Monika Ward, PhD.

Another first for the 2022 Conference is the inaugural Amelar Lecture "Men's Reproductive Health and the Environment: Declining Sperm Counts are only the Tip of the Iceberg" by Dr. Shanna Swan. This lecture is sponsored by the Richard D. Amelar Endowment Fund initiated followed Dr. Amelar's passing in 2020.

As always, the latest work by our attendees is on display in several poster and oral sessions, including "Flash Talks" picked from selected abstract submissions, designed to stimulate thoughtful discussion and interaction among colleagues. Planned luncheons, such as the Mentoring luncheon, and Women in Andrology luncheon, provide informal presentations and networking opportunities within key interest groups.

The Annual Conference is also our opportunity to celebrate and recognize four outstanding members of our society.

Congratulations to the recipient of our Distinguished Andrologist Award, Dr. Jacquetta Trasler, Dr. George L. Gerton for the Distinguished Service Award, and the 2022 Matthew P Hardy Young Andrologist Award to Dr. Brian Hermann. We will also recognize Dr. Steven Schrader as the inaugural recipient of the ASA Impact Award. Congratulations to all of our highly deserving honorees!

As we all look forward to gathering together for the first time in 3 years, the location and climate for our conference could not be better! In May we fully expect the seasonal weather - generally warm, dry, and sunny days - that La Jolla and the greater San Diego is known for.

Our conference is headquartered at Estancia La Jolla Resort & Spa in La Jolla, California, a California-coastal rancho setting on 10 acres conveniently located between the two quaint seaside villages of La Jolla and Del Mar and adjacent to world-class Torrey Pines Golf Course. Estancia La Jolla Resort & Spa offers a range of upscale accommodations from charming rancho style deluxe rooms to expansive suites with world-class facilities and exceptional service.

During the conference, please visit our exhibitors who bring us the latest technologies, supplies, and pharmaceuticals and who provide generous support for our meeting. Also please stop by the Endowment and Development Committee table to learn about the many ways our endowment funds support the society. Thank you to everyone who has made a gift this year; there is still time to help support our Annual Fund goal for 2022-23.

Be sure to use the Whova app or Whova desktop access for the latest updates on the Conference and to plan and organize your agenda.

Finally, throughout the spring we will closely monitor local and national public health conditions and advise attendees accordingly regarding COVID-19 health and safety requirements. These will also be updated on the conference website, so please keep a close eye these developments in the upcoming months.

I hope you will enjoy our wonderful conference and leave with new ideas and collaborations that will transform your research and clinical practice in Andrology. I look forward to seeing you in sunny La Jolla!

Sincerely,

Michael A. Palladino, Ph.D. Immediate Past President, American Society of Andrology

PROGRAM COMMITTEE

James M. Hotaling, MD, MS, FECSM (Co-Chair)
Salt Lake City, Utah

Jon M. Oatley, PhD (Co-Chair)
Pullman, Washington

Jim Smith, MD
Lafayette, California

Polina Lishko, PhD
Berkeley, California

Wei Yan, MD, PhD
Long Beach, California

Tessa Lord, PhD
Dudley, Australia

Mary Samplaski, MD
La Canada Flintridge, California

Akansha Mehta, MD
Atlanta, Georgia

AMERICAN SOCIETY OF ANDROLOGY

***ANDROLOGY* EXECUTIVE OFFICE**

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EXECUTIVE OFFICE

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NOTICE TO READERS

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

GENERAL MEETING INFORMATION

WELCOME TO LA JOLLA

La Jolla is a hilly, seaside neighborhood within the city of San Diego, California occupying 7 miles of coastline along the Pacific Ocean.

Located about 20 minutes north of downtown, La Jolla is home to the wide, white-sand beaches of La Jolla Shores with access to activities, including surfing, snorkeling, paddleboarding, kayaking, and more!

In addition to the prime beaches, La Jolla is also home to cultural stops such as the La Jolla branch of the Museum of Contemporary Art San Diego and local art galleries where you can see out-of-the box paintings by the late Theodore Geisel, the longtime La Jolla resident better known as Dr. Seuss.

Estancia La Jolla Hotel and Spa is located in the Torrey Pines area of La Jolla. It is across the street from the University of California San Diego (USCD) campus and renowned Salk Institute. It is a short distance from Torrey Pines State Reserve, La Jolla Shores, downtown La Jolla and University Town Center.

For more information and suggested La Jolla activities, visit https://www.visitcalifornia.com/places-to-visit/la-jolla/.

HOTEL INFORMATION

Estancia La Jolla Hotel & Spa
9700 N. Torrey Pines Road La Jolla, California 92037 Main: (855) 318-7602
Website: https://www.estancialajolla.com/

TRAVEL AND TRANSPORTATION

Airport Information

The Estancia La Jolla Hotel and Spa is 15 miles or 30 minutes from San Diego International Airport (SAN) and downtown San Diego.

Taxi Cab Services

Many companies provide taxicab service at San Diego International Airport. If you need a taxi, simply follow the signs leading to the Transportation Plazas. A Customer Service Representative will place you with the first available taxi, unless you specify a particular taxicab company. Taxi rates for a one- way to La Jolla are approximately $30-$40.

Rental Car Information

All car rental pick-ups and drop-offs for rental car companies serving San Diego International Airport occur at the Consolidated Rental Car Center. Free dedicated shuttle busses run continually to ferry customers between the airport terminals and the Rental Car Center.

Parking

Valet parking at the hotel is $45 per night and self-parking is $35 per night. The hotel is pleased to offer guests complimentary EV charging at on-site Tesla stations through valet parking.

Ride Share

Several companies offer Ride Share services at San Diego International Airport. Travelers can be dropped off curbside at both Terminal 1 and Terminal 2.
Designated Ride Share passenger pickup locations are listed below:

Terminal 1: Exit baggage claim via the escalators, walk across the Sky Bridge toward Ground Transportation and the parking lot, exit down the escalators toward the parking lot, and turn right to the Transportation Plaza. Use crosswalk to the third lane for pick-up area. See signage for assistance.

Terminal 2: Upon exiting baggage claim, use main crosswalk to Transportation Plaza. Ride sharing pick-up is located in the second lane on the right. See signage for assistance.

GENERAL MEETING INFORMATION

REGISTRATION/INFORMATION DESK HOURS

Testis Workshop

Location: La Jolla Ballroom

Wednesday, May 4, 2022	6:00 p.m. - 8:30 p.m.
Thursday, May 5, 2022	7:00 a.m. - 6:00 p.m.
Friday, May 6, 2022	7:00 a.m. - 6:00 p.m.
Saturday, May 7, 2022	7:15 a.m. - 12:00 p.m.

ASA Annual Conference

Location: La Jolla Ballroom

Friday, May 6, 2022	2:00 p.m. - 6:00 p.m.
Saturday, May 7, 2022	7:00 a.m. - 7:00 p.m.
Sunday, May 8, 2022	6:30 a.m. - 6:30 p.m.
Monday, May 9, 2022	6:30 a.m. - 6:30 p.m.
Tuesday, May 10, 2022	7:00 a.m. - 12:30 p.m.

OPTIONAL WORKSHOPS

ASA Andrology Lab Workshop*

"The Emerging Technologies in Laboratory Testing for Male Reproduction"

Date: May 7, 2022
Time: 8:30 a.m. - 5:00 p.m.
Location: Learning Theater
Cost: $450 for ASA members (active or trainee),
$475 for nonmembers

EVENING FUNCTIONS

Testis Workshop Welcome Reception

Date: Wednesday, May 4, 2022
Time: 8:15 p.m. - 9:30 p.m.
Location: La Jolla Ballroom Foyer
Cost: One ticket included in registration fee

ASA Annual Meeting Welcome Reception

Join us for a welcome reception to connect with friends and colleagues.

Date Saturday, May 7, 2022
Time: 7:30 p.m. - 9:00 p.m.
Location: La Jolla Ballroom
Dress: Business casual or casual attire
Cost: *One ticket included in ASA registration*;
$50 for additional tickets.

ASA Trainee Forum and Mixer

Date: Sunday, May 8, 2022
Time: 7:30 p.m. - 9:00 p.m.
Location: Garden Courtyard
Cost: Complimentary; all members of the society are welcome.

The ASA Trainee Forum and Mixer provides an opportunity for trainee members to meet other trainees, as well as more established members of the society. This is a relaxed, informal event with appetizers, beer, and wine provided. Senior members of the society will be present in this informal forum and discussion group setting to answer your questions about relevant topics such as grant writing, searching for a post-doctor job, alternative PhD career paths, succeeding in the clinic or lab, etc.

ASA Annual Banquet

Date: Monday, May 9, 2022
Time: 7:00 p.m. - 12:00 a.m.
Location: TBD
Cost: $100 for attendees (members/nonmembers), $60 for trainees
Includes dinner and entertainment. Please sign up for this event on the registration form.

LUNCHEONS

Mentoring Luncheon

"Navigating Career Paths in Andrology"

Sponsored by the Diversity & Trainee Affairs Committees

Date: Sunday, May 8, 2022
Time: 12:45 p.m. - 2:15 p.m.
Location: Grande Room
Academic Perspective: Ricardo Pimenta Bertolla, DVM, PhD
HCLD Perspective: Dolores Lamb, PhD
Non-Academic Perspective: TBD
Cost: $45 for attendees (members/nonmembers), $25 for trainees

Women in Andrology Luncheon

Sponsored by the Women in Andrology Committee

Date: Monday, May 9, 2022
Time: 12:00 p.m. - 1:30 p.m.
Location: Grande Room
Host: Maria Christina W. Avellar, PhD
Cost: $45 for attendees (members/nonmembers), $25 for trainees

WELCOME FROM ASA PRESIDENT

Dear Meeting Participants,

It is with great pleasure and anticipation that I welcome you to the 47th ASA Annual Meeting, which will be held May 7-10, 2022, at the Estancia La Jolla Star and Resort, in San Diego, California, which is preceded by the XXVIth North American Testis Workshop, May 4-7, 2022.

And what a welcome it will be! After two years of virtual events, we will once again be able to meet in-person! As I write this letter, I can already hear the xylophone bell calling us to the coffee-break … where most of our networking and conversations between sessions usually takes place!

I would particularly like to thank Dr. Michael Palladino (ASA Immediate Past President) for leading the preparation and organization of this Conference, as well as Program Co-Chairs Dr. Jon Oatley and Dr. James Hotaling — and their Committee Members — for putting together a well thought out scientific program with stimulating and authoritative speakers, talks and topics on the overarching theme they have chosen for this year: "The Journey of Male Fertility: From Embryo to Adult and Back Again."

I join with Dr. Palladino in calling your attention to the excellent opportunities that will be available to all participants from the beginning of the conference to the end in the form of lectures, symposia, workshops, luncheons, oral communications, and other activities, including this year's Andrology Laboratory Workshop on "The Emerging Technologies in Laboratory Testing for Male Reproduction." It will be four days of events that should provide the highest quality basic, clinical, and translational science on male reproductive health.

It will also be a time to congratulate all of our colleagues receiving awards, and to welcome two new additions to our scientific program — the Amelar Lecture and the Impact Award.

I would like to extend a special thank you to all of the speakers who will be sharing their knowledge and expertise with us. We are also grateful for the support of our industry, technology, and pharmaceutical partners. Please take a moment to stop by and check out their exhibits and materials.

I want to reiterate that I am proud of how resilient and proactive the entire leadership team at the ASA has been in overcoming difficult challenges and continuing to serve our organization with grace under pressure in this "COVID era." Likewise, our management company partners at Veritas have performed creatively in answer to a wide range of sometimes difficult circumstances. Despite the challenges posed by the pandemic outbreak and in this almost post-pandemic "new normalcy," everyone has responded in the most effective way to make this meeting come together and to ensure its success. My sincere thanks to all who made this possible.

We know that the young members are the future of the Society. The relatively small size of the ASA and the breadth of topics presented at the Annual Meeting facilitate interaction among all members and stimulate new ideas in the field. Thus, I encourage trainees and junior scientists and clinicians to introduce yourself to the more experienced members who are present at the meeting, thereby creating opportunities to learn new methodologies, more deeply understand and evaluate data and perhaps even open up a job opportunity or two. The coffee breaks, poster and panel discussions, and social events, provide other fruitful opportunities to interact as well. Bring your ideas and energy to our annual meeting!

Are you a new or prospective member of the Society? Please consider joining the ASA and participating more actively with us.

Most of all, our fellow members, thank you for your participation. It is because of your enthusiastic involvement that the ASA is able to host a successful meeting. So, help us to continue our legacy of success!

I look forward to an excellent meeting with great scientists coming from different countries around the world to share new and exciting results.

I truly hope attendees will enjoy the conference and have a wonderfully memorable experience in La Jolla.

Welcome and enjoy the conference.

Sincerely,

Maria Christina W. Avellar
President, American Society of Andrology

EMIL STEINBERGER MOMORIAL LECTURE

(Supported by the Emil Steinberger Endowment Fund)

Bradley Cairns, PhD
University of Utah School of Medicine

Dr. Bradley Cairns received his B.S. (Honors) in Chemistry from Lewis and Clark College in Portland, Oregon in 1987. He conducted his graduate work at Stanford with Nobel Laureate Roger Kornberg PhD on both signal transduction and chromatin remodeling, where he was the first to purify a chromatin remodeling complex (SWI/SNF complex). He received his PhD in Cell Biology from Stanford in 1996, and also conducted an early phase of postdoctoral training (funding from the American Cancer Society). Dr. Cairns received formal postdoctoral training with Fred Winston PhD in the Department of Genetics at Harvard Medical School (funding from the Leukemia Society of America), where he continued to study chromatin remodeling complexes, including RSC and SWI/SNF. In 1998, he joined the faculty of the Department of Oncological Sciences and the Huntsman Cancer Institute where he continued studies on chromatin remodeling mechanisms, and initiated new work on germline and early embryo chromatin and transcription mechanisms. In 2000, he was appointed as an Investigator with the Howard Hughes Medical Institute. He is now Professor and Chair of the Department of Oncological Sciences and is the Jon and Karen Huntsman Presidential Professor of Cancer Research and the Chief Academic Officer at the Huntsman Cancer Institute - both within the University of Utah, School of Medicine. He was elected to the American Academy of Arts and Sciences in 2017.

The Cairns lab has made many contributions to understanding chromatin structure and gene expression principles in the germline – which include: 1) The first genome-wide analyses of histone retention and modifications in human spermatozoa, revealing the retention in mature sperm of differentially modified histones enriched at the promoters of developmental and housekeeping genes. 2) Evidence that pluripotency network genes are silenced in spermatogonia by chromatin and DNAme, and the surprising result that gametogenesis in mice and humans involves the transcription of hundreds of genes bearing DNA methylation and co-incident H3K4me3 (Hammoud et al., Cell Stem Cell, 2014). 3) The first single-cell analysis of human spermatogonia and characterization of four distinct developmental states (Guo et al., Cell Stem Cell, 2017). 4) Identification of the most undifferentiated spermatogonial state, termed State 0, and its presence from infant stages through adulthood (Guo et al., Cell Research, 2018). 4) Characterization of spermatogonia and somatic cell co-development during puberty, and exploration of roles for testosterone by examining testes from testosterone-suppressed transfemales (Guo et al., Cell Stem Cell, 2020) and 5) Evidence that State 0 stem cells emerge in the fetus, and exploration of the specification of fetal somatic niche cells (Guo et al., Cell Stem Cell, 2021). Most recently, the lab collaborated to explore the impact of aging and high BMI on testis GSCs and somatic cells, revealing major alterations in somatic cell function (Nie et al., bioRxiv 2021).

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.

Jacquetta Trasler, MD, PhD
McGill University Health Centre

Jacquetta Trasler is a Distinguished James McGill Professor in the Departments of Pediatrics, Human Genetics and Pharmacology & Therapeutics at McGill University and a Senior Scientist at the Research Institute of the McGill University Health Centre (RI-MUHC). She received her MD and PhD degrees from McGill University followed by postdoctoral training in reproductive molecular biology at Tufts University in Boston.

Dr. Trasler's internationally recognized research in the field of reproductive and developmental epigenomics has provided important discoveries relating to the normal development of children and the prevention of birth defects. Her authority in this area reflects her early entry into the fields of molecular biology and epigenetics, her steadfast implementation of innovative methodologies, and her excellence in communicating the challenges and opportunities of this field to her colleagues.

The epigenome provides a way for the environment to interact with our genomes to modify gene expression in ways that are both adaptive and maladaptive and undergoes its most dramatic remodeling in germ cells and early embryos. Dr. Trasler's clinical focus is the 10-20% of couples who suffer from infertility or delay child-bearing and increasingly (1-6% of pregnancies) resort to the use of assisted reproductive technologies (ART). Both infertility and the use of ART are associated with epigenetic (DNA methylation) defects and adverse outcomes in children. Her epigenomic research stands out in considering sex-specific effects along with the impact of both fathers' and mothers' exposures to environmental stressors or diet in predisposing their children to developmental defects, metabolic disease, obesity, and neurodevelopmental disorders. Dr. Trasler's group has constantly integrated new state-of-the-art methodology into their epigenomics research. She has been continuously funded by the Canadian Institutes of Health Research (CIHR) as well as numerous other funding agencies, has established a rich network of local, National, and International collaborations and helped colleagues establish epigenomics-related research projects and funding. She remains passionate about teaching and training the next generation of researchers; this is evident from her teaching commitments, the success of her research trainees, the many graduate student committees she is on, her leadership role in mentoring clinician-investigators and her career-long involvement with the McGill MD-PhD Program. She has been an inspiration to many of her colleagues who span multiple generations in our discipline and beyond.

Dr. Trasler has served the field of Andrology extensively throughout her career, and she has been a long-time active member of the American Society of Andrology. Her service to the ASA includes as

Co-Editor, 1st Edition Handbook of Andrology, Chair of Women in Andrology, Council Member, Secretary and Executive Council Member, Chair of the Postgraduate Course, frequent Member of the Annual Meeting Program Organizing Committee, and member of the Editorial Board of Journal of Andrology. In addition, she has been an Executive Committee Member and Program Chair of the North American Testis Workshop, President of the Canadian Fertility and Andrology Society, and contributed as a member of the Scientific Program Committee of the International Society of Andrology, the National Academy of Sciences, Engineering and Medicine (USA) Committee on Generational Effects of Serving in the Gulf War, NIH Advisory Committees and as Standing Member of NIH Peer review committee CMIR.

Amongst Dr. Trasler's leadership roles are Director of the McGill University MD-PhD Program, Scientific Officer and Chair of the CIHR Endocrinology Peer Review Committee, Scientific Director of the Montreal Children's Hospital Research Institute, Deputy Director/Chief Scientific Officer of the RI-MUHC, Member of the Institute Advisory Board for the CIHR Institute of Genetics, and Member of the CIHR Stem Cell Oversight Committee.

In recognition of her exceptional scientific contributions to the field of Andrology, outstanding leadership roles in the ASA and broader scientific community, and commitment to advancement of science in male reproductive health, the American Society of Andrology is pleased to award its highest honor, the Distinguished Andrologist Award to Dr Jacquetta Trasler.

DISTINGUISHED ANDROLOGISTS	
1976	Roy O. Greep & M.C. Chang
1977	Robert E. Mancini
1978	Robert S. Hotchkiss
1979	Thaddeus Mann
1980	John MacLeod
1981	Alexander Albert
1982	Eugenia Rosemberg
1983	Kristen B.D. Eik-Nes
1984	Mortimer B. Lipsett
1985	Robert H. Foote
1986	Alfred D. Jost
1987	Emil Steinberger
1988	Yves W. Clermont
1989	C. Alvin Paulsen
1990	Marie-Claire Orgebin-Crist
1991	Philip Troen
1992	C. Wayne Bardin
1993	Anna Steinberger
1994	Richard J. Sherins
1995	Rupert P. Amann
1996	J. Michael Bedford
1997	Brian P. Setchell
1998	Ryuzo Yanagimachi
1999	Richard D. Amelar
2000	Bayard T. Storey
2001	Frank S. French
2002	Geoffrey M. H. Waites
2003	David M. de Kretser
2004	Ronald Swerdloff
2005	Mitch Eddy
2006	Norman Hecht
2007	Eberhard (Ebo) Nieschlag
2008	Bernard Robaire
2009	William Bremner
2010	Dolores Lamb
2011	Barry Zirkin
2012	Erwin Goldberg
2013	Christina Wang
2014	Gail S. Prins
2015	Deborah A. O'Brien
2016	Barry T. Hinton
2017	Masaru Okabe
2018	John McCarrey
2019	Terry T. Turner
2020	Vassilios Papadopoulos
2021	R. John Aitken

DISTINGUISHED SERVICE AWARD

(Supported by the Past Presidents Endowment Fund)

Dr. George Gerton, PhD

Perlman School of Medicine at the University of Pennsylvania

From 1984 until his retirement in 2021, George L. Gerton, Ph.D. served as Research Professor in Reproductive Biology of the Perelman School of Medicine at the University of Pennsylvania. He received a B.A. with Honors in Biochemistry and Molecular Biology from the University of California at Santa Barbara in 1975 and a Ph.D. in Biochemistry from the University of California at Davis in 1980. As a postdoctoral scientist, he first worked at Tufts-New England Medical Center and then did research at Harvard Medical School where he developed his expertise in spermatogenesis and sperm structure and function. Over the course of his career, his research team investigated the biochemistry, cell biology, and molecular biology of male reproduction, fertilization, preimplantation embryo development, and environmental health. His laboratory made key discoveries relating to acrosomal and flagellar proteins of sperm, fertilization mechanisms, and the effects of endocrine disruptors on reproduction. He also served in administrative positions in PENN's Center for Research on Reproduction and Women's Health and the Center of Excellence in Environmental Toxicology. His funding was derived from public agencies, including the National Institute of Child Health and Human Development and the National Institute of Environmental Health Sciences, as well as private foundations.

Dr. Gerton has been a member of ASA since 2001, serving in several roles. He is an Immediate Past President of the Society and served as Vice President, President, and a member of the ASA Executive Council. As ASA President, he led the Society through the important transition to a new management company, and he also coordinated activities through the first year of the COVID-19 pandemic, including the ASA's first all virtual meeting. These contributions, driven by his passion to sustain and elevate ASA operations, were key to ensuring

the cohesiveness and vitality of ASA during this very challenging time. His legacy is enduring.

Amongst other contributions to the ASA, Dr. Gerton has actively participated in and/or chaired many committees including Trainee Affairs, Membership, Constitution and By-Laws, Ethics, Endowment and Development, Diversity, Nominating, Program, and Archives and History. He has worked to expand the diversity of the ASA by providing opportunities for trainees from underrepresented minorities to participate and become members of the ASA, combining his passion for trainee advocacy with his interest in promoting the careers of underrepresented minorities in andrology. He continues to facilitate greater opportunities for clinicians and basic scientists to collaborate on meaningful research projects, integrate trainees and new members deeper into the workings of the society, develop programs to bolster the careers of trainees and members from diverse backgrounds, expand membership to include a broader base of workers in andrology, and seek ways to reach out to the public and legislators to make "andrology" a commonly used term and spread the word that male reproductive health is an important issue in need of more funding for research and clinical care.

In recognition of his extraordinary service to the American Society of Andrology, and to the field of Andrology in general, his dedication to and exceptional leadership that have improved ASA functions, and his effective actions that have built connections between ASA members, the American Society of Andrology is pleased to present the Distinguished Service Award to Dr. George Gerton.

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 P. 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
2018	Rudi Ansbacher
2019	Wylie C. Hembree
2020	Douglas T. Carrell
2021	Patricia S. Cuasnicu

MATTHEW P. HARDY YOUNG ANDROLOGY AWARD

(Supported by the Matthew P. Hardy 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.

Brian P. Hermann, PhD

University of Texas at San Antonio

Dr. Hermann is currently an Associate Professor in the Department of Neuroscience, Developmental and Regenerative Biology at the University of Texas at San Antonio.

Dr. Hermann received his PhD in 2005 from the University of Kansas Medical Center under the supervision of Leslie Heckert where he studied transcriptional regulation of the FSH-receptor and Steroidogenic factor-1 genes. He then completed postdoctoral training in lab of Dr. Kyle Orwig at the Magee-Womens Research Institute, University of Pittsburgh where he performed the first definitive spermatogonial stem cell (SSC) transplantation in non-human primates. With support from an NIH K99/R00 award, Dr. Hermann started his lab at the University of Texas at San Antonio (UTSA) in 2011. The Hermann lab studies the mechanisms regulating SSC fate in adults during steady-state spermatogenesis, SSCs fate specification during testicular development, and potential SSC-based therapeutic strategies for male infertility. He has generated some of the first scRNAseq data relating to the mammalian testis presented it to the field in a readily accessible format that enabled many other research teams to benefit from his expertise. Dr. Hermann also directs the UTSA Genomics Core, which specializes in single-cell and spatial methodologies, and has applied those approaches to understanding the identity of SSCs and their regulatory framework.

Dr. Hermann's authority in area of scRNAsequence and new genomics tools is reflected in the publication of four chapters relating to these in 2021 alone. In addition, he has been engaged in securing several grants relating not only to discerning control elements in spermatogenesis, but also to addressing the pathogenesis of encephalomyelitis and Lyme disease and developing tools for understanding brain development.

He has been an active member of the Communications and Public Affairs Committee since 2016 and now serves as its Chair.

Dr. Hermann's work has been lauded by colleagues around North American and beyond. He has recently been named as a Fulbright US Senior Scholar to undertake research on spermatogenesis and early embryo development in livestock species with the Physiology of Reproduction group at the University of Murcia in Spain as the recipient of this prestigious position.

The diversity and depth of Brian's contributions Andrology is indeed impressive, and the ASA is delighted to name him as the 2022 Matthew P. Hardy ASA Young Andrologist.

MATTHEW P. HARDY YOUNG ANDROLOGIST AWARD RECIPIENTS	
1982	L.J.D. Zaneveld
1983	William B. Neaves
1984	Lonnie D. Russell
1985	Bruce D. Schanbacher
1986	Stephen J. Winters
1987	Ilpo T. Huhtaniemi
1988	Larry Johnson
1989	Barry T. Hinton
1990	Luis Rodriguez-Rigau
1991	Patricia M. Saling
1992	Gary R. Klinefelter
1993	Robert Chapin
1994	Wayne J.G. Hellstrom
1995	Christopher DeJonge
1996	Paul S. Cooke
1997	Gail A. Cornwall
1998	William R. Kelce
1999	Stuart E. Ravnik
2000	Matthew P. Hardy
2001	Jacquetta Trasler
2022	Christopher L.R. Barratt
2003	Joanna E. Ellington
2004	Kate Loveland
2005	Janice Bailey
2006	Janice P. Evans
2007	John K. Amory
2008	Moira K. O'Bryan
2009	Michael A. Palladino
2010	Peter Liu
2011	Humphrey Yao
2012	Wei Yan
2013	Jacques J. Tremblay
2014	Sarah Kimmins
2015	Jon M. Oatley
2016	Lee B. Smith
2017	Polina V. Lishko
2018	Michael L. Eisenberg
2019	Mariano G. Buffone
2020	James M Hotaling
2021	Clemence Belleannee

IMPACT AWARD

(Supported by the Eugenia Rosemberg Endowment Fund)

Dr. Steven Schrader, PhD

Cincinnati, OH

Dr. Steven Schrader led the Reproductive Health Assessment Team for the National Institute for Occupational Safety and Health (NIOSH), an agency of the Centers for Disease Control and Prevention (CDC), from 1983 until his retirement in 2017.

Dr. Schrader received his BS (1974), MS (1975), and PhD (1978) from the University of Missouri, undertook postdoctoral training at University of Miami, and was an assistant professor at Roosevelt University in Chicago. Upon joining NIOSH 1983, he established the male reproductive health assessment program and thereby enabled numerous occupational field investigations into exposure impacts which included ethylene dibromide, glycol ethers, lead, nickel, military radar, and numerous pesticides. Dr. Schrader's work evaluating sexual function in bicycle police officers led to expanded research in this area for male and female bicyclists. His efforts have inspired many researchers to address how other aspects of occupational hazards affect male sexual function, and thereby providing a framework for evidence-based policy formation.

Dr. Schrader's authority has been recognized by invitations to present his research nationally and internationally, and his research program has involved collaborations in reproductive research with universities, other federal, and international agencies. This included speaking to the United Nations Commission on Sustainable Development on how endocrine disrupter chemicals can adversely impact reproductive health of men. Dr. Schrader served on the WHO workgroup that published the 5th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. He served on the CDC workgroup that prepared the *National Public Health Action Plan for the Detection, Prevention and Management of Infertility.*

Dr. Schrader's research goal in occupational studies was to have objective numerical measures that could withstand statistical rigor. There are many examples of the international impact of his research. Dr. Schrader developed a male reproductive health profile for conducting occupational field studies which he and his team used in workplaces across the United States, Canada, and Russia. He conducted a longitudinal study semen quality evaluating the within and between variation of the various semen measures. He developed an early computer digitizing method to assess sperm motility and morphometry, many of these measures are being used in current CASA machines. He became an advocate and instructor of process quality control in the Andrology Lab. During the emerging HIV epidemic in the 1980s, Dr. Schrader published *Safety Guidelines for the Andrology Laboratory*. In recognition of this work, he has received the Alice Hamilton Science Award for Occupational Safety and Health four times and the Bullard-Sherwood Research-to-Practice (r2p) Award. In 2017, Dr. Schrader was honored with the NIOSH Lifetime Scientific Achievement Award.

Dr. Schrader has been active in the American Society of Andrology since 1983. He has made over 60 scientific presentations at the ASA meetings. He has served on the ASA Executive Council, including as Secretary, and repeatedly on the Annual Meeting Program Committee, in 2018 as Program Co-Chair. He served on the Nominating and Endowment Committees. Dr. Schrader was very active in the Andrology Laboratories and Archives and History Committees, and he has chaired them both while also contributing as a faculty member for numerous Andrology Laboratory Workshops. In 2014, Dr. Schrader coordinated a joint meeting of the leadership of the ASA and CDC to determine common interests and establish mechanisms of interaction between them. In 2015, he led the "40 and Forward Celebration" to mark the ASA's 40th anniversary. This extensive and impressive service to the ASA was recognized in 2015, when Dr. Schrader received the ASA Distinguished Service Award.

ASA has benefited from Dr. Schrader's long-standing and active engagement with the ASA, including his activities that have enabled many of our members and annual scientific conference delegates to

make their own contributions to the field of Andrology. However, his impact extends well beyond the ASA, as evidenced from his other activities and award. In light of his career-long devotion to developing and implementing strategies that protect enhance male reproductive health, and his dedicated intellectual contributions and advocacy leading to practical outcomes of relevance to Andrology, the ASA is proud to award the inaugural ASA Impact Award to Dr. Steven Schrader.

AUA LECTURE

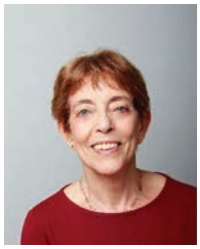
(Sponsored by the American Urological Association)

Craig Niederberger, MD
University of Illinois at Chicago

Dr. Craig Niederberger is Clarence C. Saelhof Professor and Head of the Department of Urology in the College of Medicine at the University of Illinois at Chicago and holds a joint appointment as Professor in the Department of Bioengineering in the College of Engineering. He was Co-Editor in Chief of Fertility and Sterility and a prior section editor for the Journal of Urology. He is a primary editor of the fourth and upcoming fifth editions of Infertility in the Male. He served as general program chair for the American Society for Reproductive Medicine's annual meeting in Atlanta in 2009 and has served as president of the Society for the Study of Male Reproduction within the American Urological Association and as president of the Society for Male Reproduction and Urology within the American Society for Reproductive Medicine.

AMELAR LECTURE

(Supported by the Richard D. Amelar Endowment Fund)



Shanna Swan, PhD
University of Rochester

Dr. Shanna Swan is an Environmental and Reproductive Epidemiologist and Professor of Environmental Medicine and Public Health at the Icahn School of Medicine at Mount Sinai, New York. Her work examines the impact of environmental exposures on reproductive health and neurodevelopment. Since 1998, Dr. Swan has been conducting multicenter pregnancy cohort studies which include more than 1500 mothers and their children followed from birth, to better understand how prenatal and early childhood exposure to stressors impact reproductive health and neurodevelopment. In 2017, Dr. Swan. Dr. Levine and colleagues published "Temporal Trends in Sperm Count: a systematic review and meta-regression analysis," which received worldwide media coverage and led to her writing: *Count Down*: *How Our Modern World Is Threatening Sperm Counts, Altering Male and Female Reproductive Health, and Imperiling the Future of the Human Race* (Scribner, 2021), which is currently being translated into nine languages.

EDUCATIONAL NEEDS, OBJECTIVES, AND ACCREDITATION

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 "Single Cell Testis Atlas," "Increasing Diversity in the Biomedical Workforce: What, Why and How," "Sperm Chromatin and Proteomic Contribution to the Zygote," "Return of a Forgotten Hero – *Zfy* Roles in Male Reproduction," "New Insights on Male Precocious Puberty," "The Future of Andrology," "Are Sperm Counts Declining Worldwide?" During the plenary sessions, attendees will have the opportunity to participate in question-and-answer sessions.

TARGETAUDIENCE

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

ASA 47th ANNUAL CONFERENCE

EDUCATIONAL NEEDS

Male reproductive health is a complex of biological and physiological processes and disease states are a major societal issue. Although decades of research studies have yielded seminal information about how male reproduction is controlled at the cellular and molecular levels, significant gaps in understanding still exist and effective strategies to solve infertility and urological diseases are limited. To address these issues, urologists, basic and clinician scientists need to share information on topics such as developmental biology, regenerative medicine, and reproductive technology development. The 2022 American Society of Andrology Annual Meeting is designed to provide a forum for engagement and collaboration amongst clinicians and scientists working at the cutting-edge of male reproductive health.

Basic researchers will learn from clinicians about the current state of patient needs for solving male reproductive health problems. Concordantly, clinicians will learn from basic researchers about recent novel discoveries in mechanisms regulation male reproductive function and the cutting-edge of diagnostics and reproductive technology development.

EDUCATIONAL OBJECTIVES

At the conclusion of the ASA 44th Annual Conference, attendees will be able to:

1. Discuss the current state of male contraceptive development
2. Understand how sperm genetic content influences embryo development and offspring health
3. Describe how sperm quality is influenced by age and environmental factors
4. Understand the risk factors associated with paternal age on genomic integrity of sperm.
5. Associate genetic deficiencies with abnormalities in meiotic recombination and sperm aneuploidy
6. Identify metabolic disorders that could lead to male infertility
7. Understand how breakdown in perinatal development of the germline can manifest as infertility.

ACCREDITATION

Credit Statements

Saturday: 1.25 | Sunday: 7.75 | Monday: 5.75 | Tuesday: 3.50

Category 1

Amedco LLC designates this **live activity** for a maximum of **18.25** *AMA PRA Category 1 Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Accreditation Statement

In support of improving patient care, this activity has been planned and implemented by Amedco LLC and Ohio Urological Society (OUS). Amedco LLC 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.

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INTERPROFESSIONAL CONTINUING EDUCATION

SCHEDULE OF EVENTS

THE XXVITH NORTH AMERICAN TESTIS WORKSHOP *Not CME Accredited*

"New Horizons in Testis Biology and Men's Health"
May 4 – 7, 2022 | Estancia La Jolla Hotel & Spa | La Jolla, CA
Chair: Wei Yan, MD, PhD & Vice Chair: Kate Loveland, PhD
All sessions will be located in ***La Jolla Ballroom*** unless otherwise noted.
Speakers and times are subject to change.

WEDNESDAY, MAY 04, 2022

6:00 p.m. - 8:30 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:00 p.m. - 7:15 p.m. **Welcome and Opening Remarks**
Program Chair: Wei Yan, MD, PhD
The Lundquist Institute at Harbor-UCLA/UCLA

7:15 p.m. - 8:15 p.m. **KEYNOTE ADDRESS:**
Where Are We in the Development of Non-Hormonal Male Contraceptives?
Martin M. Matzuk, MD, PhD
Baylor College of Medicine

8:15 p.m. - 9:30 p.m. **Testis Workshop Welcome Reception**

THURSDAY, MAY 05, 2022

7:00 a.m. - 6:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:15 a.m. - 8:00 a.m. **Continental Breakfast**

8:00 a.m. - 8:55 a.m. **BENCHMARK LECTURE I**

8:00 a.m. - 8:05 a.m. **Introduction**
Chair: Kate Loveland, PhD
Monash University and Hudson Institute

8:05 a.m. - 8:55 a.m. **Lumicrine Factors in the Regulation of Spermatogenesis**
Masahito Ikawa, PhD
Osaka University

SESSION I: NEW KIDS ON THE BLOCK: NOVEL FACTORS THAT REGULATE SPERMATOGENESIS (1)

8:55 a.m. - 9:00 a.m. **Introduction to Session I**
Chairs: Erwin Goldberg, PhD
Northwestern University
Lauren Chukrallah, PhD Candidate
Rutgers University

9:00 a.m. - 9:40 a.m. **Everything Old is New Again: Redefining Protamine Structure and Function**
Sue Hammoud, PhD
University of Michigan

9:40 a.m. - 10:20 a.m. **Identification of a Novel Germ Cell Granule with Potential Roles in Meiotic Germ Cell Translation**
Elizabeth Snyder, PhD
Rutgers University

10:20 a.m. - 10:40 a.m. **Break**

10:40 a.m. - 11:20 a.m. **Pachytene piRNAs in Germ Cell Development**
Chen Chen, PhD
Michigan State University

11:20 a.m. - 11:35 a.m. **Short Talk #1**
TENT4A and TENT4B Control Poly(A) Tail Length and Non-A Contents in Murine Spermatogenic Cells
Presented By: Zhuqing Wang, PhD
The Lundquist Institute at Harbor UCLA

11:35 a.m. - 11:50 a.m. **Short Talk #2**
In Pro-Spermatogonia the RNA Binding Protein DND1 Targets Transcripts for Epigenetic, Cell Cycle, and Translation Regulation
Presented By: Victor Ruthig, PhD
Duke University Medical Center

11:50 a.m. - 1:10 p.m. **Lunch (on your own)**

SESSION II: NEW KIDS ON THE BLOCK: NOVEL FACTORS THAT REGULATE SPERMATOGENESIS (2)

1:10 p.m. - 1:15 p.m. **Introduction to Session II**
Session Chairs: Jon Oatley, PhD
Washington State University
Tessa Lord, PhD
University of Newcastle

1:15 p.m. - 1:55 p.m. **New Ideas on Fertility Preservation for People with Testes**
Kyle Orwig, PhD
University of Pittsburgh

1:55 p.m. - 2:35 p.m. **What Spermatogonial Stem Cells Can Tell Us About Molecular Networks, Infertility, and Selfish DNA**
Miles Wilkinson, PhD
University of California, San Diego

2:35 p.m. - 2:55 p.m. **Break**

2:55 p.m. - 3:35 p.m. **Sperm-like Stem Cell-mediated Genome Editing**
Jinsong Li, PhD
Chinese Academy of Sciences

3:35 p.m. - 3:50 p.m. **Short Talk #3**
A Master Transcription Factor Influencing Both Pro-Spermatogonia and SSC Differentiation
Presented By: Kun Tan, PhD
University of California, San Diego

3:50 p.m. - 4:05 p.m. **Short Talk #4**
Embryonic Origin of Testicular Macrophages
Presented By: Andreas Meinhardt, PhD
Justus-Liebig University of Giessen

4:05 p.m. - 6:00 p.m. **Poster Session I**
Location: Pacifica Ballroom

FRIDAY, MAY 06, 2022

7:00 a.m. - 6:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:15 a.m. - 8:00 a.m. **Continental Breakfast**

8:00 a.m. - 8:55 a.m. **BENCHMARK LECTURE II**

8:00 a.m. - 8:05 a.m. **Introduction**
Chair: Michael Griswold, PhD
Washington State University

8:05 a.m. - 8:55 a.m. **Why is the Sperm Proteome Modified in the Female Reproductive Tract?**
Mariana Wolfner, PhD
Cornell University

SESSION III: BACK TO THE BASICS: REGULATION OF SPERMATOGENESIS (1)

8:55 a.m. - 9:00 a.m. **Introduction to Session III**
Chairs: Erika Matunis, PhD
Johns Hopkins University
Hayden McSwiggin, PhD
The Lundquist Institute at Harbor UCLA

9:00 a.m. - 9:40 a.m. **New Insights into Sertoli Cell Fate Determination and Maintenance**
Humphrey Yao, PhD
NIEHS, Research Triangle, NC

9:40 a.m. - 10:20 a.m. **Epigenetic Regulation of Spermatogonial Stem Cell Specification**
John McCarrey, MS, PhD
University of Texas, San Antonio

10:20 a.m. - 10:40 a.m. **Break**

10:40 a.m. - 11:20 a.m. **Some Assemply Required: How to Build a Meiotic DNA-Breaking Machine**
Scott Keeney, PhD
Sloan Kettering Cancer Center, New York

11:20 a.m. - 11:35 a.m. **Short Talk #5**
Spatial Dynamics of Protein Translation in Sertoli Cells
Presented By: Ana Cristina Lima, PhD
Oregon Health & Sciences University

11:35 a.m. - 11:50 a.m. **Short Talk #6**
Chromatin States During Spermatogonial Differentiation
Presented By: Christine Schleif, Master Graduate Student
Washington State University

11:50 a.m. - 1:10 p.m. **Lunch (on your own)**

SESSION IV: BACK TO THE BASICS: REGULATION OF SPERMATOGENESIS (2)

1:10 p.m. - 1:15 p.m. **Introduction to Session IV**
Chairs: Qi Chen, MD, PhD
University of California - Riverside
Kien Tran
University of Pittsburgh

1:15 p.m. - 1:55 p.m. **Novel Transcription Factors Regulating Spermatogenesis**
Prabhakara Reddi, PhD
University of Illinois Urbana-Champaign

1:55 p.m. - 2:35 p.m. **Testosterone Recovery Therapy Targeting Dysfunctional Leydig Cells**
Vassilios Papdopoulos, PharmD, PhD
University of Southern California, Los Angeles

2:35 p.m. - 2:55 p.m. **Break**

2:55 p.m. - 3:35 p.m. **Follicle-Stimulating Hormone and D-cyclins Regulate Male Germ Cell Maintenance**
T. Rajendra Kumar, PhD
University of Colorado

3:35 p.m. - 3:50 p.m. **Short Talk #7**
Leydig Cell Regeneration in Adult Mouse Testes
Presented By: Jasmin Jeffrey, BS, PhD Candidate
Johns Hopkins University School of Medicine

3:50 p.m. - 4:05 p.m. **Short Talk #8**
Higher Incidence of Embryonic Defects in Offspring Conceived by Assisted Reproduction from Fathers with Sperm Epimutations
Presented By: Gurbet Karahan, PhD
McGill University

4:05 p.m. - 6:00 p.m. **Poster Session II**
Location: Pacifica Ballroom

SATURDAY, MAY 07, 2022

7:15 a.m. - 12:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:15 a.m. - 8:00 a.m. **Continental Breakfast**

8:00 a.m. - 8:55 a.m. **BENCHMARK LECTURE III**

8:00 a.m. - 8:05 a.m. **Introduction**
Chair: Monika Ward, PhD
University of Hawaii

8:05 a.m. - 8:55 a.m. **Paternal Epigenome and Offspring Health**
Jacquetta Trasler, MD, PhD
McGill University

SESSION V: ON TOP OF GENETICS – TESTICULAR EPIGENOME AND OFFSPRING HEALTH

8:55 a.m. - 9:00 a.m. **Introduction to Session V**
Chairs: Wenfeng An, PhD, MPH
South Dakota State University
Jasmin Jeffrey, PhD Candidate
Johns Hopkins University

9:00 a.m. - 9:40 a.m. **Sperm-mediated Transgenerational Inheritance**
Corrado Spadafora, PhD
National Research Council of Italy

9:40 a.m. - 10:20 a.m. **Sperm RNA-mediated Epigenetic Inheritance**
Isabelle Mansuy, PhD
University and ETH Zurich

10:20 a.m. - 10:40 a.m. **Break**

10:40 a.m. - 11:20 a.m. **Short and Long-term Effects of Male Germline Reprogramming in the absence of TET1**
Marisa Bartolomei, PhD
University of Pennsylvania

11:20 a.m. - 11:35 a.m. **Update From the NIH**
Thaddeus Schug, PhD
National Institute of Environmental Health Sciences

11:35 a.m. - 11:50 a.m. **Concluding Remarks and Acknowledgements**
Wei Yan, MD, PhD
The Lundquist Institute at Harbor-UCLA

11:50 a.m. - 12:00 p.m. **Announcement of the XXVIIth North American Testis Workshop**
Kate Loveland, PhD
Monash University and Hudson Institute

12:00 p.m. **Adjournment**

SCHEDULE OF EVENTS

The American Society of Andrology 47th Annual Conference

"The Journey of Male Fertility: From Embryo to Adult and Back Again"

May 7 - 10, 2022 | Estancia La Jolla Hotel & Spa | La Jolla, CA

Program Chairs: James Hotaling, MD & Jon Oatley, PhD

All sessions will be located in the ***La Jolla Ballroom*** unless otherwise noted. Speakers and times are subject to change.

FRIDAY, MAY 06, 2022

2:00 p.m. - 6:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

SATURDAY, MAY 07, 2022

7:00 a.m. - 7:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

4:00 p.m. - 9:30 p.m. **Exhibits Open**
Location: La Jolla Ballroom

8:30 a.m. - 5:00 p.m. **ASA Andrology Lab Workshop* "The Emerging Technologies in Laboratory Testing for Male Reproduction"**
Location: Learning Theater
*See page 22 for full ALW schedule **ALW schedule included in separate attachment**

6:00 p.m. - 6:20 p.m. **President's Welcome**
Maria Christina W. Avellar, PhD
ASA President

6:20 p.m. - 7:30 p.m. **EMIL STEINBERGER MEMORIAL LECTURE: Using Single Cell Genomics to Explore Testis Development, Spermatogonia and Spermatogenesis**
Supported by the Emil Steinberger Endowment Fund
Introducer: Michael A. Palladino, PhD
Bloomfield College
Speaker: Brad Cairns, PhD
University of Utah School of Medicine

7:30 p.m. - 9:00 p.m. **ASA Welcome Reception**
Location: La Jolla Ballroom

SUNDAY, MAY 08, 2022

6:30 a.m. - 8:00 a.m. **Past Presidents' Breakfast**
Location: Garden Suite

6:30 a.m. - 6:30 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 4:00 p.m. **Exhibits Open**
Location: La Jolla Ballroom

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

8:00 a.m. - 8:15 a.m. **ASA Distinguished Andrologist Award**
Supported by the Eugenia Rosemberg Endowment Fund
**Not CME Accredited*
Introducer: Gail Cornwall, PhD
Texas Tech University Health Sciences Center
Recipient: Jacquetta Trasler, MD, PhD
McGill University

8:15 a.m. - 8:30 a.m. **ASA Distinguished Service Award**
Supported by the ASA Past Presidents Endowment Fund
**Not CME Accredited*
Introducer: Michael A. Palladino, PhD
Bloomfield College
Recipient: George Gerton, PhD
Perlman School of Medicine at the University of Pennsylvania

8:30 a.m. - 8:45 a.m. **ASA Impact Award**
Supported by the Eugenia Rosemberg Endowment Fund
**Not CME Accredited*
Introducer: Susan Rothmann, PhD, HCLD
Fertility Solutions, Inc.
Speaker: Steven Schrader, PhD
Cincinnati, OH

8:45 a.m. - 9:45 a.m. **DIVERSITY LECTURE: Increasing Diversity in the Biomedical Workforce: What, Why and How**
Supported by the ASA Education Endowment Fund
Introducer: Peter Y. Liu, MBBS (Hons I), PhD, FRACP
The Lundquist Institute at Harbor UCLA
Speaker: Keith Norris, MD, PhD
University of California, Los Angeles

9:45 a.m. - 11:00 a.m. **Poster Session I**

11:00 a.m. - 12:45 p.m. **SYMPOSIUM I: The Aging Male Gamete: Implications for Population Health**
Moderators: Tessa Lord, PhD
University of Newcastle
Mary Samplaski, MD
University of Southern California

11:00 a.m. - 11:25 a.m. **Paternal Age Effect Mutations in the Male Germline**
Anne Goriely, PhD
University of OxforD

11:25 a.m. - 11:50 a.m. **How Can We Select Optimal Gametes from Older Fathers?**
Jim Hotaling, MD
University of Utah School of Medicine

11:50 a.m. - 12:15 p.m. **Paternal Age and Offspring Somatic Health**
Michael Eisenberg, MD
Stanford University School of Medicine

12:15 p.m. - 12:30 p.m. **Q&A**

12:30 p.m. - 12:35 p.m. **Oral Abstract**: In Vitro Spermatogenesis from Immature Testicular Tissues Cryopreserved for Pediatric Patients Before Gonadotoxic Therapy: A Step Towards Fertility Preservation and Restoration
Nagham Younis

12:35 p.m. - 12:40 p.m. **Oral Abstract**: Sperm mRNA Profiles Reflect Reproductive History in Patients Presenting for Vasectomy or Infertility Assessment
Daniel Spade, PhD

12:45 p.m. - 2:15 p.m. **Industry Sponsored Product Theater**

12:45 p.m. - 2:15 p.m. **Mentoring Luncheon**: Navigating Career Paths in Andrology
Sponsored by the Diversity and Trainee Affairs Committees

12:45 p.m. - 2:15 p.m. **Editorial Board Luncheon**

2:15 p.m. - 4:40 p.m. **SYMPOSIUM II: Sperm & Embryonic Competence and the Commitment to Meiosis**
Moderators: John McCarrey, MS, PhD
University of Texas, San Antonio
Jacquetta Trasler, MD, PhD
McGill University

2:15 p.m. - 2:40 p.m. **Epigenetic Programming and the 3D Genome Organization in Male Germ Cells**
Satoshi Namekawa, PhD
University of California, Davis

2:40 p.m. - 3:05 p.m. **DNA Methylation in Gametogenesis and Embryogenesis**
Deborah Bourchis, PhD
Curie Institute

3:05 p.m. - 3:30 p.m. **Regulation of Meiotic Entry and Progression**
Devanshi Jain, PhD
Rutgers University

3:30 p.m. - 3:55 p.m. **Regulation of Meiotic Recombination**
Paula Cohen, PhD
Cornell University

3:55 p.m. - 4:25 p.m. **Q&A**

4:25 p.m. - 4:30 p.m. **Oral Abstract**: Decoding the Molecular Deficiencies Underlying Oligoasthenoteratospermia: Identification of AARDC5 Deficiency
Mariana Giassetti, DVM, PhD

4:30 p.m. - 4:35 p.m. **Oral Abstract**: Mutation and Microdeletions in PRSS50 and LRWD1 Affect male Fertility
Hunter Flores

4:35 p.m. - 4:40 p.m. **Oral Abstract**: RBFOX2 Copy Number Variation Causes Hypospadias by Disrupting Mesenchymal to Epithelial Transition in Penis Development
Victor Ruthig, MS, PhD

4:40 p.m. - 5:00 p.m. **Break**

5:00 p.m. - 6:00 p.m. **EAA LECTURE: Sperm Chromatin and Proteomic Contribution to the Zygote**
Introducer: Patricia Cuasnicu, PhD
Instituto de Biologia y Medicina Experimental
Speaker: Rafael Olivia, PhD
University of Barcelona

6:00 p.m. - 7:30 p.m. **SYMPOSIUM III: Balancing Transparency and Privacy of Donor Gametes and Offspring**
**Organized by the Trainee Affairs Committee and supported by the CREATE Endowment Fund*
Debate Panelists –
Transparency: Lisa Campo-Engelstein, PhD
University of Texas Medical Branch
Privacy: Vincent Couture, PhD
Laval University
Industry Perspective: Molly O'Brien, Esq.
International Fertility Law Group

7:30 p.m. - 9:00 p.m. **Trainee Mixer**
Sponsored by Program Committee & Trainee Affairs Committee
Location: Garden Courtyard

MONDAY, MAY 09, 2022

6:30 a.m. - 6:00 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

7:00 a.m. - 3:30 p.m. **Exhibits Open**
Location: La Jolla Ballroom

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

8:00 a.m. - 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE: Return of a Forgotten Hero – *Zfy* Roles in Male Reproduction**
Supported by the Women in Andrology Endowment Fund
Introducer: Celia Santi, MD, PhD
Washington University School of Medicine
Speaker: Monika Ward, PhD
Institute for Biogenesis Research

9:00 a.m. - 9:15 a.m. **Break**

9:15 a.m. - 9:30 a.m. **Matthew P. Hardy Young Andrologist Award**
Supported by the Matthew P. Hardy Endowment Fund
Introducer: John McCarrey, MS, PhD
University of Texas, San Antonio
Recipient: Brian Hermann, PhD
University of Texas, San Antonio

9:30 a.m. - 9:40 a.m. **Outstanding Trainee Investigator and Trainee Awards**

9:40 a.m. - 10:40 a.m. **INTERNATIONAL LECTURE: New Insights on Male Precocious Puberty**
Supported by the ASA General Endowment Fund
Introducer: Patricia Cuasnicu, PhD
Instituto de Biologia y Medicina Experimental
Speaker: Ana Claudia Latronico, MD, PhD
Universidade de Sao Paulo

10:40 a.m. - 12:00 p.m. **Poster Session II**

12:00 p.m. - 1:30 p.m. **Lunch on Own**

12:00 p.m. - 1:30 p.m. **Women in Andrology Luncheon**
Sponsored by the Women in Andrology Committee
Location: Grande Room
Host: Celia Santi, MD, PhD
Washington University School of Medicine

1:30 p.m. - 3:00 p.m. **SYMPOSIUM IV: Embryonic and Fetal Origins of the Male Germline**
Moderators: Miles Wilkinson, PhD
University of California, San Diego
Brian Hermann, PhD
University of Texas, San Antonio

1:30 p.m. - 1:55 p.m. **Embryonic Origins of the Germline**
Amander Clark, PhD
University of California, Los Angeles

1:55 p.m. - 2:20 p.m. **Androgen Receptor Signaling and Endocrine Disruption in the Fetal Propermatogonial Development**
Diana Laird, PhD
University of California, San Francisco

2:20 p.m. - 2:45 p.m. **Testis Formation in the Human Fetus**
Jingtao Guo, PhD
University of Utah School of Medicine

2:45 p.m. - 3:00 p.m. **Q&A**

3:00 p.m. - 3:20 p.m. **Break**

3:20 p.m. - 5:00 p.m. **SYMPOSIUM V: The Intersection of Metabolism and Male Reproductive Health**
Moderators: Michael Eisenberg, MD
Stanford University School of Medicine
Tracy Clement, PhD
Texas A&M University

3:20 p.m. - 3:45 p.m. **Leptin Signaling in Male Reproduction**
Gwen Childs, PhD
University of Arkansas Medical Sciences

3:45 p.m. - 4:10 p.m. **Modulation of Spermatogonial Stem Cell Activity by Hypoxia and Hypoxia-Driven Metabolic Pathways**
Tessa Lord, PhD
University of Newcastle

4:10 p.m. - 4:35 p.m. **Metabolite Control over Mouse Sperm Motility**
Chris Geyer, PhD
East Carolina University

4:35 p.m. - 4:50 p.m. **Q&A**

4:50 p.m. - 4:55 p.m. **Oral Abstract**: Causative Influence of Tcte1 Knockout on Energetic Chain Transportation, Apoptosis and Spermatogenesis – Implications for Male Infertility
Marta Olszewska, MS, PhD

4:55 p.m. - 5:00 p.m. **Oral Abstract**: Investigation of the Immune Milieu of the Epididymis: Strategic Positioning of Leukocytes
Andreas Meinhardt, PhD

5:00 p.m. - 5:30 p.m. **National Institute of Health Updates**

5:00 p.m. - 5:15 p.m. **NICHD Update**
Travis Kent
National Institutes of Child Health and Human Development

5:15 p.m. – 5:30 p.m. **NIEHS Update**
Thaddeus Schug, PhD
National Institute of Environmental Health Sciences

5:30 p.m. - 6:30 p.m. **ASA Annual Business Meeting**

7:00 p.m. – 12:00 a.m. **ASA Annual Banquet**
Location: Pacifica Ballroom

TUESDAY, MAY 10, 2022

7:00 a.m. - 8:00 a.m. **2023 Program Committee Meeting**
Location: Dracena Room

7:00 a.m. - 12:30 p.m. **Registration/Information Desk Open**
Location: La Jolla Ballroom Foyer

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

8:00 a.m. - 9:00 a.m. **AUA LECTURE: The Future of Andrology**
Sponsored by the AUA Educational Grant
Introducer: Jim Hotaling, MD
University of Utah School of Medicine
Speaker: Craig Niederberger, MD
University of Illinois at Chicago

9:00 a.m. - 9:15 a.m. **Poster Awards**

9:15 a.m. - 9:30 a.m. **Break**

9:30 a.m. - 10:30 a.m. **AMELAR LECTURE: Are Sperm Counts Declining Worldwide?**
Supported by the Richard D. Amelar Endowment Fund
Introducer: Susan Rothmann, PhD, HCLD
Fertility Solutions, Inc.
Speaker: Shanna Swan, PhD
University of Rochester

10:30 a.m. - 12:00 p.m. **SYMPOSIUM VI: Debate: Hormonal vs Non-Hormonal Approaches to Male Contraception**
Moderators: John Amory, MD, MPH, MSc
University of Washington
Wipawee "Joy" Winuthayanon, BSN, PhD
Washington State University

10:30 a.m. - 11:00 a.m.	**Non-Hormonal** Wei Yan, MD, PhD *The Lundquist Institute at Harbor-UCLA*
11:00 a.m. - 11:30 a.m.	**Hormonal** Stephanie Page, MD, PhD *University of Washington*
11:30 a.m. - 11:35 a.m.	**Rebuttal – Non-Hormonal**
11:35 a.m. - 11:40 a.m.	**Rebuttal – Hormonal**
11:40 a.m. - 11:50 a.m.	**Q&A**
11:50 a.m. - 11:55 a.m.	**Oral Abstract**: Targeting the MEIG1/PACRG Interaction for Male Contraceptive *Wei Li, MD*
11:55 a.m. - 12:00 p.m.	**Oral Abstract**: High-Throughput Drug Screening Method for Discovering Novel CatSper Inhibitors *Guillermina Luque, PhD*
12:00 p.m. - 12:15 p.m.	**Closing Remarks**

SATURDAY, MAY 07, 2022

Andrology Lab Workshop

"The Emerging Technologies in Laboratory Testing for Male Reproduction"

8:30 a.m. - 8:45 a.m.	**Continental Breakfast / Check-In / Introduction to ALW**
8:45 a.m. - 9:15 a.m.	**Basic Semen Analysis in Evaluation of Male Infertility – WHO 2021 New Guidelines with Limitations of CASA Technology** Anna-Marie Bort, MLT (ASCP)^{CME} Charles Muller, PhD, HCLD
9:15 a.m. - 9:45 a.m.	**Clinical and Functional Relevance of Sperm Morphology** Charles Muller, PhD, HCLD
9:45 a.m. - 10:00 a.m.	**Break**
10:00 a.m. - 11:30 a.m.	**Interactive Workshop: Sperm Morphology** (with exercises and quiz) Anna-Marie Bort, MLT (ASCP)^{CME} Angela Reese, TS Susan Kinser, BS
11:30 a.m. - 12:00 p.m.	**Advanced Semen Testing in WHO 2021 – Role of Oxidative Stress and Sperm DNA Damage Evaluation** Suresh Sikka, PhD, HCLD, CC (ABB) Manesh Kumar Panner Selvam, PhD
12:00 p.m. - 1:15 p.m.	**Lunch Break and Guest Lecture Cytogenomics and Male Infertility: Macroscope to Microscope** Introducer: Anna Chockalingam, DVM, PhD, HCLD (ABB) (Genetic Consultant) Speaker: V.G. Dev, PhD, FACMG (Medical Director, Genetics Associates Inc, Founding member of ACMG)
1:15 p.m. - 2:30 p.m.	**Interactive Workshop: Genetic Evaluation of Male Infertility – A Cytogenetic Approach** (with exercises and quiz) Anna Chockalingam, DVM, PhD, HCLD (ABB) (Genetic Consultant) Jesse Gore MS, CG (ASCP)
2:30 p.m. - 3:00 p.m.	**Omics Technologies in Diagnosis and Management of Male Infertility** Manesh Kumar Panner Selvam, PhD Saradha Baskaran, PhD
3:00 p.m. - 3:15 p.m.	**Break**
3:15 p.m. - 3:45 p.m.	**Erectile Dysfunction: Diagnosis and Treatment** Jim Hotaling, MD, MS, FECSM
3:45 p.m. - 4:45 p.m.	**Interactive Workshop: SOP for Duplex Ultrasound in Assessment of Erectile Dysfunction** (with exercises and quiz) Suresh Sikka, PhD, HCLD, CC (ABB)
4:45 p.m. – 5:00 p.m.	**Feedback from Attendees and Adjourn**

ASA SPEAKER ABSTRACTS

SATURDAY, MAY 7, 2022
6:20 pm – 7:30 pm

EMIL STEINBERGER MEMORIAL LECTURE

Single Cell Testis Atlas

Brad Cairns, PhD
University of Utah School of Medicine

The human testis is a complex organ with distinct phases of prenatal and postnatal development. The testis seminiferous tubule harbors germline development and differentiation, which includes spermatogonial development and spermatogenesis, guided by somatic cells, which provide distinct environments and signaling processes. Diverse studies in rodents, primates, and human tissue have together greatly advanced our understanding. However, until recently a deep understanding of these processes at the molecular and genomic level in humans has been lacking.

We have conducted a deep molecular and genomic understanding of human testis development and gametogenesis through single-cell genomics, complemented by chromatin analyses and protein validation, and my lecture will highlight the insights provided. In 2017, we published the first single-cell analysis of male germline stem cells (GSCs) and characterized four distinct human adult GSC states along their development. Next, we identified the most undifferentiated GSC, termed State 0, and showed its presence from birth through adulthood. Third, we characterized GSC and somatic cell co-development during puberty, and explored roles for testosterone by examining testes from testosterone-suppressed transfemales. Fourth, we determined the emergence of State 0 GSCs in the fetus, and the specification of fetal somatic niche cells. Most recently, we explored the impact of aging and high BMI on testis GSCs and somatic cells, revealing major alterations in somatic cell function. Taken together, our work – combined with the work of others – has revealed how modern genomics approaches have advanced our understanding of testis development and spermatogenesis, providing a foundation for advances in clinical management of infertility.

SUNDAY, MAY 8, 2022
8:45 am – 9:45 am

DIVERSITY LECTURE

Increasing Diversity in the Biomedical Workforce: What, Why and How

Keith Norris, MD, PhD
University of California, Los Angeles

Nearly 70 years after the supreme court ruling on Brown vs. Board of Education affirming all Americans should have equal public education and more than 50 years after the passing of the Civil Rights Bill, many Americans from racial and ethnic minority groups and women continue to face barriers to entry and/or ascent in the biomedical sciences. Numerous national reports have called for improving America's leadership in scientific research and diversifying the scientific workforce to foster innovation and reduce racial and ethnic disparities in health.

Almost every governmental and professional organization has created diversity statements, diversity committees and promises for change, to little avail. Across all science disciplines these entities have created an array of "pipeline" programs, mostly short-term training opportunities for persons underrepresented in science and medicine (URM) at various levels in their career. However, these have not led to expected gains in diversifying student entry into biomedical careers with some dropping out at different transition points along the career pathway, termed the "leaky URM pipeline."

This presentation will touch upon systemic barriers and why our existing approaches on a whole have failed. Strategies for the way forward include inclusive science practices, true education reform, and the need for a change to an equity-minded consciousness to overcome the root causes of inequity in resources and opportunity that perpetuate underrepresentation of targeted racial and ethnic groups as well as health disparities for these same groups.

SUNDAY, MAY 8, 2022
11:00 am – 11:25 am

SYMPOSIUM I: The Aging Male Gamete: Implications for Population Health

Paternal Age Effect Mutations in the Male Germline

Anne Goriely, PhD
University of Oxford

It is now well-established that our genomes mutate at a slow but constant rate of ~60 new point mutations per generation. Although most new point mutations (> 80%) originate from the father and increase in frequency at the rate 1-2 mutation/paternal year, very little is known about the cellular mechanisms that allow the adult testis to reconcile the contradictory demands for abundant sperm production over many decades and a low mutation rate across generations.

We have previously described a process where some pathogenic mutations hijack the homeostatic mechanisms of sperm production to their own advantage. This 'selfish selection' mechanism was originally proposed to explain the paternal age-effect and high birth prevalence of some Mendelian disorders, such as Apert syndrome (*FGFR2*) or

achondroplasia (*FGFR3*). It relies on principles similar to oncogenesis to explain why these mutations occur spontaneously at levels up to 1000-fold higher than the background rate. Importantly, this process emphasizes the intimate link that exists between sperm production/fertility, germline mutation rate and ageing.

I will summarize our current understanding of de novo mutations, their importance for human disease and genome heterogeneity and how paternal age influences their pattern of occurrence. I will then describe the concept of selfish selection and some of the novel strategies we are developing to study de novo mutations directly within human testes. Finally, I will speculate on the broader implications of this process and the importance of the regulation of spermatogenesis for human disease, genome diversity and evolution.

SUNDAY, MAY 8, 2022
11:25 am – 11:50 am

SYMPOSIUM I: The Aging Male Gamete: Implications for Population Health

How Can We Select Optimal Gametes from Older Fathers?

Jim Hotaling, MD
University of Utah School of Medicine

Paternal age has increased significantly over the last few decades. Along with this, we have also seen an increase in the rates of autism fueled, in part, by advanced paternal age, which is known to be associated with higher sperm de novo mutation rates. Currently, we have no way to select sperm with lower de novo mutation rates. While groups have been able to sequence individual spermatozoa to determine the mutation rate, it is impossible to do this without destroying the sperm. We will cover available technologies that might have the potential to select optimal sperm. While none of these technologies are currently available for routine clinical practice it is highly likely that they will be in the next 5-7 years. We will also cover what the impact of this would be on the field and how this could change reproductive health.

SUNDAY, MAY 8, 2022
11:50 am – 12:15 pm

SYMPOSIUM I: The Aging Male Gamete: Implications for Population Health

Paternal Age and Offspring Somatic Health

Michael Eisenberg, MD
Stanford University School of Medicine

With paternal age rising in the US and around the world, implications to the children have received increasing attention. Data suggesting higher risks of health conditions. In addition, neuropsychiatric effects of children with older fathers are coming into focus and suggest areas for further consideration.

SUNDAY, MAY 8, 2022
2:15 pm – 2:40 pm

SYMPOSIUM II: Sperm & Embryonic Competence and the Commitment to Meiosis

Epigenetic Programming and the 3D Genome Organization in Male Germ Cells

Satoshi Namekawa, PhD
University of California, Davis

The gene expression program of germ cells is distinct from that of somatic lineages. Importantly, the somatic gene expression program is largely suppressed in male germ cells. Instead, male germ cells retain a unique cellular identity that is passed on to sperm and gives rise to a totipotent zygote after fertilization. Our research seeks to understand the molecular basis for the unipotent developmental program in the germline and the recovery of totipotency in the next generation of life. We have ascertained critical roles for Polycomb-based mechanisms in the establishment of a unique epigenome in male germ cells, i.e., mechanisms that preprogram unipotent germ cell differentiation. At the mitosis-to-meiosis transition, we have begun to reveal dynamic epigenetic programming based on cellular memories established in progenitor cells. In this talk, I will present our recent findings as to how the 3D genome organization is preprogrammed to direct spermatogenic differentiation, undergoing dynamic epigenetic programming to prepare the next generation.

SUNDAY, MAY 8, 2022
2:40 pm – 3:05 pm

SYMPOSIUM II: Sperm & Embryonic Competence and the Commitment to Meiosis

DNA Methylation in Gametogenesis and Embryogenesis

Deborah Bourc'his, PhD
Curie Institute

DNA methylation plays a critical role in spermatogenesis, as evidenced by the male sterility of DNA methyltransferase (DNMT) mutant mice. Previous mutations in DNMT3C and the DNMT3L co-factor have highlighted the key role for this modification in silencing retrotransposons and preventing their activity to interfere with meiosis. We have now found a striking division of labor in the establishment of the methylation landscape of male germ cells during fetal development and its functions in spermatogenesis: while DNMT3C is essential for methylating the promoters evolutionarily young and active retrotransposons—that represent only 1% of the genome—, DNMT3A broadly methylates the

rest of the genome: intergenic sequences, gene bodies, and old and inactive retrotransposons. Lack of DNMT3A-dependent methylation is also incompatible with fertility; however, the defect resides here in loss of spermatogonial stem cell (SSC) plasticity, not a meiotic catastrophe. By reconstructing developmental trajectories through single-cell RNA-seq and by profiling chromatin states, we found that *Dnmt3A* mutant SSCs can only self-renew and no longer differentiate, in association with spurious enhancer activation that enforces an irreversible stem cell gene program. We therefore provide a novel function for DNA methylation in male fertility: the epigenetic programming of SSC commitment to differentiation and to life-long spermatogenesis supply.

SUNDAY, MAY 8, 2022
3:05 pm - 3:30 pm

SYMPOSIUM II: Sperm & Embryonic Competence and the Commitment to Meiosis

Genome Integrity During Gametogenesis

Devanshi Jain, PhD
Rutgers University

Meiosis is the process by which a diploid cell gives rise to haploid gamete cells and is essential for sexual reproduction. This cell division program is driven by a specialized transcriptome, which supports complex chromosome behaviors that are integrated with cell cycle progression. Although general meiotic principles are conserved, those driven by mammalian-specific or poorly conserved genes are challenging to identify and have limited our understanding of mammalian meiosis. We employed a phenotype-driven forward-genetics screen in mouse to explore the genetic basis of mammalian meiotic processes and isolated several novel mutants with diverse and dramatic meiotic phenotypes. I will present our screen along with studies characterizing one affected gene, encoding the RNA helicase YTHDC2.
We and others recently identified YTHDC2 to be a critical regulator of meiotic entry in the mouse germline, and demonstrated that YTHDC2, along with its binding partner and functional collaborator MEIOC, constitutes a post-transcriptional control pathway that switches cells from mitotic to meiotic gene expression programs. In this talk, I will present our surprising insights into the mechanisms of how this pathway shapes the gene expression landscape during gametogenesis to regulate both entry into and progression through the meiotic cell cycle.

SUNDAY, MAY 8, 2022
3:30 pm – 3:55 pm

SYMPOSIUM II: Sperm & Embryonic Competence and the Commitment to Meiosis

Regulation of Meiotic Recombination

Paula Cohen, PhD
Cornell University

During meiotic prophase I, formation of DNA double strand breaks and their subsequent repair as noncrossovers or crossovers (COs) involves highly conserved mechanisms that ensure that homologous chromosomes pair, become physically connected and then segregate equally at the first meiotic division. Errors in these processes result in the formation of eggs and sperm bearing the wrong number of chromosomes, resulting in fetal loss and birth defects. Such errors are highly prevalent in human meiosis. In many organisms, at least two pathways exist to ensure that appropriate CO numbers are achieved. The major class I pathway involves heterodimers of the DNA mismatch repair family of MutS homologs 4/5 (MSH4/MSH5: MutSγ) and MutL homologs 1/3 (MutLγ). However, previous studies in our lab have shown that MutSγ accumulation in mouse far exceeds the total number of class I COs, while a point mutation within the ATPase domain of MSH5 results in loss of all COs across the genome, not just those of the class I variety. Moreover, loss of the class II pathway, which is mediated by the MUS81-EME1 endonuclease, results in a compensatory increase in class I events, leading to normal CO frequency. Thus, we hypothesize that mammalian meiosis is chracterized by a co-ordinated DNA damage response following DSB induction, and that this response involves integration between the class I and class II machineries. Ongoing studies in our lab are focused on understanding how CO outcomes are ensured to achieve highly regulated CO frequency and distribution at the end of prophase I.

SUNDAY, MAY 8, 2022
5:00 pm – 6:00 pm

EAA LECTURE

Sperm Chromatin and Proteomic Contribution to the Zygote

Rafael Oliva, PhD
Additional authors: Meritxell Jodar, Judit Castillo
University of Barcelona

The majority of the chromatin present in mature spermatozoa consists in highly compact toroidal nucleoprotamine structures formed by positively charged protamines coupled to the negatively charged DNA. These structures organize about 92% of the sperm DNA in the human species, and there is evidence that they function condensing and protecting the sperm chromatin into a compact and hydrodynamic nucleus. In addition, nucleohistone complexes representing about 8% of the DNA of the sperm cell also organize the male genome and are strategically located coupled specific genomic sites and genes. While the histones, histone variants and protamines represent the most abundant sperm chromatin proteins, proteomic analysis has led to the identification of over 500 additional chromatin associated proteins. Histone modifiers, DNA methylases, zinc fingers, transcription factors and structural proteins have been identified in the spermatozoan chromatin specifically associated either to the nucleohistone or to the nucleoprotamine regions. Integrative analysis of the human sperm cell proteome with that of the oocyte and blastocyst, has identified a

subset of specific sperm proteins with potential roles in early embryo development. Furthermore, posttranslational modifications of the histones, protamines and additional chromatin proteins, such as methylation, phosphorylation and acetylation contribute with additional layers of epigenetic information. Overall, the evidence suggests that the sperm chromatin contributes with a very complex epigenetic structure and information to the embryo, and that its alterations may be responsible for some of the male infertility cases and/or the epigenetic transmission of altered phenotypes. Supported by "Ministerio de Ciencia e Innovación," Spain (PI20/00936).

MONDAY, MAY 9, 2022
8:00 am – 9:00 am

WOMEN IN ANDROLOGY LECTURE

Return of a Forgotten Hero – *Zfy* Roles in Male Reproduction

Monika Ward, PhD
Institute for Biogenesis Research

The Y chromosome encoded zinc finger protein gene, *ZFY*, has once been in the center of attention as a potential candidate for the testis-determining factor. When the fame went to another Y chromosome gene, *SRY*, *ZFY* was quickly forgotten, and it has taken more than two decades for it to re-emerge with newly ascribed spermiogenic roles. The mouse Y chromosome has two *Zfy* copies, *Zfy1* and *Zfy2*, both with potential to act as a transcription factor and with postnatal expression restricted to spermatogenic cells. Using mice with severe Y chromosome deficiencies and supplementing *Zfy* transgenes, we, and others, revealed many interesting aspects of *Zfy* function. *Zfy* were shown to play spermatogenic quality functions during meiosis I, promote the second meiotic division, facilitate spermatid elongation, enhance the efficiency of round spermatid injection (ROSI) in the context of limited Y chromosome gene contribution, and enable transformation of round spermatids into sperm functional in assisted fertilization. Although the evidence regarding importance of *Zfy* was clear, interpretation of findings required acknowledging that altered expression or loss of Y chromosome genes other than *Zfy* might have contributed to observed spermiogenic phenotypes. To address it we recently developed and characterized *Zfy* knockout mice and have shown that loss of *Zfy* led to infertility and severe spermatogenic defects. In my talk today, I will present the current state of knowledge regarding the roles mouse *Zfy* play in male reproduction, with a focus on newest findings with *Zfy* knockout mice. Supported by NIH HD072380 and HCF 17CON-86294.

MONDAY, MAY 9, 2022
9:40 am – 10:40 am

INTERNATIONAL LECTURE

New Insights on Male Precocious Puberty

Ana Claudia Latronico, MD, PhD
Sao Paulo Medical School, Sao Paulo University, Brazil

Pubertal timing is regulated by the complex interplay of genetic, environmental, nutritional and epigenetic factors. Central precocious puberty (CPP) is defined as the premature reactivation of the hypothalamic-pituitary-gonadal axis, starting before the ages of 8 and 9 years in girls and boys, respectively. To date, four monogenic causes of CPP have been described. Loss-of-function mutations in Makorin Ring Finger Protein 3 (MKRN3), a maternally imprinted gene on chromosome 15 within the Prader-Willi syndrome locus, are the most common identified genetic cause of CPP. More recently, several mutations in a second maternally imprinted gene, Delta-like noncanonical Notch ligand 1 (DLK1), have also been associated with CPP. Mutations in the genes encoding kisspeptin (KISS1) and its receptor (KISS1R), potent activators of GnRH secretion, have also been described in association with CPP, but remain rare monogenic causes. CPP has both short- and long-term health implications for children, highlighting the importance of understanding the mechanisms contributing to early puberty. In boys, CPP is usually caused by brain structural lesions, including congenital and acquired lesions. Recently, we studied 20 boys from 17 families with idiopathic CPP. All of them had normal brain magnetic resonance imaging. Eight boys from five families harbored four distinct heterozygous MKRN3 mutations predicted to be deleterious for protein function, p.Ala162Glyfs*14, p.Arg213Glyfs*73, p.Arg328Cys and p.Arg365Ser. One boy carried a previously described KISS1-activating mutation (p.Pro74Ser). The frequency of MKRN3 mutations among these boys with idiopathic CPP was significantly higher than previously reported female data (40% vs. 6.4%, respectively, $p < 0.001$). Boys with MKRN3 mutations had typical clinical and hormonal features of CPP. Notably, they had later pubertal onset than boys without MKRN3 abnormalities (median age 8.2 vs. 7.0 years, respectively, $p = 0.033$). We demonstrated a high frequency of MKRN3 mutations in boys with CPP, previously classified as idiopathic, suggesting the importance of genetic analysis in this group. The boys with CPP due to MKRN3 mutations had typical features of CPP, but with puberty initiation at a borderline age.

MONDAY, MAY 9, 2022
1:30 pm – 1:55 pm

SYMPOSIUM IV: Embryonic and Fetal Origins of the Male Germline

Embryonic Origins of the Germline

Amander Clark, PhD
University of California, Los Angeles

In the adult testis, germline stem cells called Spermatogonial stem cells are responsible for the continual production of sperm a process called stem cell self-renewal and differentiation. Loss of Spermatogonial stem cells from the seminiferous tubule epithelium of the testis results in Sertoli Cell Only Syndrome, a cause of male infertility. Spermatogonial stem cells are not specified in the testis during seminiferous tubule formation. Instead, Spermatogonial stem cells originate from embryonic progenitor germ cells which are set-aside at the time of embryo implantation, well before the embryonic testis begins to

develop. Therefore, abnormalities in progenitor germ cell formation and localization to the testis before birth is an alternate cause Sertoli Cell Only Syndrome and infertility. In order to understand the cell and molecular mechanisms required for embryonic germ cell progenitor formation and therefore the identification of new infertility genes, my laboratory uses embryonic and induced pluripotent stem cells, human tissues as well as animal modelling to achieve this goal. Here, I will present our latest data on the cell and molecular mechanisms required to establish the earliest germ cell progenitors in the embryo called primordial germ cells (PGCs), and the importance of epigenetic regulators to coordinate the timing of PGC differentiation into a testicular germ cell fate.

MONDAY, MAY 9, 2022
1:55 pm – 2:20 pm

SYMPOSIUM IV: Embryonic and Fetal Origins of the Male Germline

Androgen Receptor Signaling and Endocrine Disruption in the Fetal Prospermatogonial Development

Diana Laird, PhD
University of California, San Francisco

Germ cells convey genetic as well as epigenetic information across generations, as supported by multiple studies of environmental insults such as exposure to endocrine disruptors, which produce measurable phenotypes in unexposed grandchildren. Phthalates are persistent chemicals known to counteract androgens and cause transgenerational defects in rodents; however, the mechanism remains unclear. Androgen receptor (AR) is well known for its function in supporting cells in the testis, but its role in fetal germ cells remains unclear. In fetal male germ cells of mice, we detected dynamic expression of AR protein which peaks at E14.5, coincident with the arrest of mitosis. Supporting the connection between AR expression and mitotic arrest in germ cells, both were delayed following a developmental exposure to DEHP. Likewise, upon conditional deletion of *Ar* in the fetal germline, we observed an increase in germ cell apoptosis and incomplete entry into mitotic arrest by E14.5. Although we found that the levels of nuclear AR in fetal germ cells could be increased with the ligand dihydroxytestosterone and decreased with its inhibitor flutamide, this does not exclude the possibility of concomitant non-classical signaling via AR. This study provides the first evidence of an autonomous function for AR in fetal germ cells and raises the possibility that endocrine-disrupting chemicals could exert direct effects on developing germ cells to alter their developmental trajectory and potentially their epigenetic landscape.

MONDAY, MAY 9, 2022
2:20 pm – 2:45 pm

SYMPOSIUM IV: Embryonic and Fetal Origins of the Male Germline

Testis Formation in the Human Fetus

Jingtao Guo, PhD
University of Utah School of Medicine

Human testis development in prenatal life involves complex changes in germline and somatic cell identity. To better understand this process, we profiled and analyzed ~32,500 single-cell transcriptomes of testicular cells from embryonic, fetal and infant stages. Our data shows that at 6-7 weeks post-fertilization as the testicular cords are established, the Sertoli and interstitial cells originate from a common heterogeneous progenitor pool, which then resolves into fetal Sertoli cells (expressing tube-forming genes) or interstitial cells (including Leydig-lineage cells expressing steroidogenesis genes). Almost 10 weeks later, beginning at 14-16 weeks post-fertilization, the male primordial germ cells exit mitosis, downregulate pluripotent transcription factors and transition into cells that strongly resemble the 'State 0' spermatogonia originally defined in the infant and adult testes. Therefore, we termed these fetal spermatogonia 'State f0'. Overall, we reveal multiple insights into the coordinated and temporal development of the embryonic, fetal and postnatal male germline together with the somatic niche.

MONDAY, MAY 9, 2022
3:20 pm – 3:45 pm

SYMPOSIUM V: The Intersection of Metabolism and Male Reproductive Health

Leptin Signaling in Male Reproduction

Gwen Childs, PhD
University of Arkansas Medical Sciences

Healthy reproduction requires adequate nutrition for the timing of puberty and other energetically expensive reproductive events. Leptin is a potent signal for nutritional status and permissive for reproduction, stimulating target cells in the hypothalamic-pituitary-gonadal (HPG) axis. This influence was first reported by studies of the appropriate timing of the neonatal leptin surge, which was vital for the normal development of cells in the HPG axis and the onset of puberty. However, serum leptin is reduced in males during puberty, likely because testosterone inhibits adipocyte leptin. In adult males, serum leptin levels are 10-50% of those in females. In vitro studies report that obesity levels of leptin inhibit Leydig or Sertoli cell functions, which may explain why leptin levels are limited.

Our studies of leptin regulation of pituitary gonadotropes reported that, whereas male mice lacking leptin receptors in gonadotropes are fertile, they have reduced levels of Gonadotropin releasing hormone receptors, serum GH, TSHβ and Prolactin, and *Fshβ*, *Cga*, *Gh* and *Ghrhr* mRNAs. We also reported that reducing serum leptin alone in obese male mice restored normal metabolic functions and reproduction. In recent single-cell RNA-seq studies of male mice on a high fat diet, we observed that gonadotropes showed significant reductions in critical regulatory genes, including *Pgr*, *Prkc3*, *Neurod4*, *Fgf41*, *Aebp1* and *Nhlh2*.

Collectively, these studies indicate that leptin levels are tightly regulated in the male to prevent inhibition of testicular function and the leptin resistance from high leptin that may compromise or reduce function of target cells in the pituitary.

Grants supporting this work: NIH R01 HD059056 (G.V.C.); NIH R01HD087057 (G.V.C. and A.M.M.); NIH R01HD093461 (A.MM., G.V.C., and M.C.M.), NIH R01DK113776-01 (G.V.C., A.M.M., and M.C.M.); NIGMS P20 GM103425 and P30GM11070 (Dr. Edgar Garcia-Rill).

MONDAY, MAY 9, 2022

3:45 pm – 4:10 pm

SYMPOSIUM V: The Intersection of Metabolism and Male Reproductive Health

Modulation of Spermatogonial Stem Cell Activity by Hypoxia and Hypoxia-driven Metabolic Pathways

Tessa Lord, PhD

University of Newcastle

Spermatogonial stem cells (SSCs) must intricately balance self-renewal with production of differentiating spermatogonia to sustain male fertility. The role of metabolic pathways in regulating stem cell function, and the intertwinement between hypoxic niches and glycolytic metabolism has been well established in other stem cell lineages, however, is poorly understood in SSCs. In exploring the transcriptome of SSCs, progenitors, and differentiating spermatogonia using scRNA-seq, we have corroborated previous studies that depict SSCs as a glycolytic cell, while demonstrating that progenitor and differentiating spermatogonia exhibit gene expression reflecting mitochondrial biogenesis and oxidative phosphorylation[1]. Further, although conflicting hypotheses exist surrounding the niche in the testis, we have demonstrated that SSCs express a suite of genes aligned with hypoxia. Additionally, assessments of *Id4-eGfp* transgenic mice[2] injected with pimonidazole demonstrated that > 80% ID4-eGFP^{Bright} SSCs exist in hypoxia, compared to < 10% of progenitors ($p < 0.001$). To further characterise the relationship between hypoxia, metabolism and overall SSC function, a germline-specific knockout mouse line has been generated for the hypoxia-responsive factor *Epas1*. Preliminary analyses suggest that proliferation of spermatogonia in these male mice may be dysregulated. A loss of EPAS1 expression has also been demonstrated with prolonged maintenance of undifferentiated spermatogonia in culture, accompanied by altered proliferation kinetics. Correspondingly, scRNA-seq analyses suggest that cultured SSCs express increased levels of mitochondrial respiratory chain assembly genes that are normally associated with progenitor spermatogonia. Understanding SSC metabolism and the downstream effects of dysregulation will be key to harnessing these cells for infertility treatments, such as those being developed for childhood cancer patients.

1. Lord T, Nixon B (2020) Metabolic changes accompanying spermatogonial stem cell differentiation. Developmental Cell 54 (4):399-411. doi:10.1016/j.devcel.2020.01.014

2. Helsel AR, Yang Q-E, Oatley MJ, Lord T, Sablitzky F, Oatley JM (2017) ID4 levels dictate the stem cell state in mouse spermatogonia. Development 144 (4):624-634. doi:10.1242/dev.146928

MONDAY, MAY 9, 2022

4:10 pm – 4:35 pm

SYMPOSIUM V: The Intersection of Metabolism and Male Reproductive Health

Metabolite Control over Mouse Sperm Motility

Chris Geyer, PhD

East Carolina University

Sperm flagella drive essential motility patterns that change during their transit through the distinct microenvironments of the female reproductive tract. In the uterus, sperm are progressively motile, with symmetrical beating of their flagella and linear movement. As sperm near the egg within the oviduct, they must change their motility during the process of capacitation. Capacitated sperm are hyperactively motile, with asymmetrical whiplash-like movements of their flagella that facilitate directional changes required for fine-tuned navigation to, and fertilization of, the egg. Only a small subset of spermatozoa in vitro successfully undergo hyperactivation. The metabolic mechanisms underpinning these essential sperm motility patterns remain poorly understood. Previous research into metabolic control over sperm motility has been focused on ATP generation, which has manifested in debates about whether sperm motility relies disproportionately on substrate-level phosphorylation of ADP in aerobic glycolysis vs oxidative phosphorylation (OXPHOS). While these ATP-generating processes are certainly essential to fuel flagellar motion and support motility, results from such studies fail to explain how such profound changes in motility appear to be driven by exposure to distinct metabolic substrates (metabolites). Therefore, expanding our understanding of sperm metabolism beyond the current focus on linear pathways is a pressing need. Here, we utilized a comprehensive sperm phenotyping workflow that utilizes parallel quantitative measurements of O_2 consumption, electron transference, and quantitative changes in live sperm motility patterns in response to specific metabolites. Our results support a model whereby specific metabolites predictably alter the NAD^+/NADH redox environment as a reversible metabolic switch to control sperm motility.

TUESDAY, MAY 10, 2022

8:00 am – 9:00 am

AUA LECTURE

The Future of Andrology

Craig Niederberger MD

University of Illinois at Chicago

Abstract not received by print date.

TUESDAY, MAY 10, 2022
9:30 am – 10:30 am

AMELAR LECTURE

Are Sperm Counts Declining Worldwide?

Shanna Swan, PhD
University of Rochester

The question "Are sperm counts declining?" was raised by a landmark paper in 1992. While widely critiqued, the overall conclusions of that paper have remained largely unchanged. Our 2017 meta-analysis (Levine 2017) reached a similar conclusion, using a far larger data set and methods (such as systematic literature review and meta-analytic techniques) developed in the interim. In this lecture I will discuss 25+ years of research on temporal trends in semen quality, and the challenges this research faces. I will describe the implications of these trends for birth rates, men's health and social and environmental risk factors that impact semen quality. I will address the challenges of studying time trends in physiologic variables; demands for rigor, attention to potential confounding and transparency of methods. For semen quality, the challenges are still greater, because of the lack of routine sample collection (unlike, e.g., cholesterol). Finally, I will argue that while uncertainties will remain in any study of changing semen quality, the dangers of failing to recognize a decline outweigh the risks of reporting an uncertain decline. Action to improve semen quality, such as decreasing unhealthy life behaviors and exposure to toxic environmental chemicals, which can have long-term, even transgenerational, impacts, can only serve to improve general health and sustainability. Therefore, I conclude that the costs of increasing our research efforts to minimize uncertainty and taking precautionary measures to reduce factors adversely affecting men's reproductive health are small compared to the cost of failing to recognize the implications of these data.

TUESDAY, MAY 10, 2022
10:30 am – 11:00 am

SYMPOSIUM VI: Debate: Hormonal vs . Non-Hormonal Approaches to Male Contraception

Non-Hormonal

Wei Yan, MD, PhD
The Lundquist Institute at Harbor-UCLA

Abstract not received by print date.

TUESDAY, MAY 10, 2022
11:00 am – 11:30 am

SYMPOSIUM VI: Debate: Hormonal vs. Non-Hormonal Approaches to Male Contraception

Hormonal

Stephanie Page, MD, PhD
University of Washington

There is a great need to expand contraceptive options for men. Unplanned pregnancy rates remain stagnant globally and many men wish to share the family planning burden. Multinational surveys of couples indicate that both male and female partners are interested in new male contraceptive methods and most women would trust their partners to use male methods. Similarly, data from participants in male hormonal contraceptive (MHC) trials is consistent with these survey findings and indicate a high degree of uptake were prototype methods to reach the marketplace. Reversible contraception available to men includes withdrawal and condoms, both are timing dependent with high typical-use failure rates. Vasectomy has high success and satisfaction rates but is considered a non-reversible method. MHC currently in development utilize testosterone or another androgen either alone or combined with progestins. Multicenter clinical efficacy trials have demonstrated conclusively that once sperm production is suppressed to near azoospermia the contraceptive efficacy of MHC is comparable to hormonal female methods. Side effects of MHC are overlap with side effects that women experience with female hormonal methods, are experienced by a minority of male users, and can be minimized with physiologic androgen dosing and progestins with limited androgen/anti-androgenic activity. Many new non-hormonal targets are undergoing preclinical toxicity and efficacy evaluation, but none has advanced to clinical trials. Reversibility, specificity and druggability have been major challenges in developing non-hormonal contraceptives but there is ongoing, exciting work towards addressing these challenges. Thus, hormonal male methods are likely to be the next novel reversible contraceptives available to men.

ASA ABSTRACT SUMMARY

Oral-1

IN VITRO SPERMATOGENESIS FROM IMMATURE TESTICULAR TISSUES CRYOPRESERVED FOR PEDIATRIC PATIENTS BEFORE GONADOTOXIC THERAPY: A STEP TOWARDS FERTILITY PRESERVATION AND RESTORATION

Nagham Younis[1,2], Shtaywy Abdalla[2], Kyle Orwig[1]
[1]Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [2]Department of Biological Sciences, University of Jordan, Amman, Jordan
Presented By: Nagham Younis, MS

Oral-2

SPERM MRNA PROFILES REFLECT REPRODUCTIVE HISTORY IN PATIENTS PRESENTING FOR VASECTOMY OR INFERTILITY ASSESSMENT

Daniel Spade[1], Gabriella Avellino[2,3], William Berg[2,3], Priyanka Bearelly[2,3], Susan Hall[1], Mark Sigman[2,3]
[1]Brown University, Providence, RI, USA, [2]Rhode Island Hospital, Providence, RI, USA, [3]Warren Alpert Medical School of Brown University, Providence, RI, USA
Presented By: Daniel Spade, PhD

Oral-3

TRANSCRIPTOMIC DIFFERENCES BETWEEN FIBROTIC AND NON-FIBROTIC TESTICULAR TISSUE REVEAL POSSIBLE KEY PLAYERS IN KLINEFELTER SYNDROME-RELATED TESTICULAR FIBROSIS

Margo Willems[1], Catharina Olsen[2], Ben Caljon[2], Yves Heremans[1], Veerle Vloeberghs[2], Jean De Schepper[2], Herman Tournaye[2], Dorien Van Saen[1], Ellen Goossens[1]
[1]Vrije Universiteit Brussel, Brussel, Belgium, [2]Universitair Ziekenhuis Brussel, Brussel, Belgium
Presented By: Margo Willems, BS, MS

Oral-4

DECODING THE MOLECULAR DEFICIENCIES UNDERLYING OLIGOASTHENOTERATOSPERMIA: IDENTIFICATION OF AARDC5 DEFICIENCY

Mariana Ianello Giassetti, Deqiang Miao, Melissa Oatley, Nate Law, Jon Oatley
Center for Reproductive Biology, School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA
Presented By: Mariana Ianello Giassetti, DVM, PhD

Oral-5

MUTATIONS AND MICRODELETIONS IN PRSS50 AND LRWD1 AFFECT MALE FERTILITY

Hunter Flores, Abhishek Seth, Juan Bournat, Liya Hu, Carolina Jorgez
Baylor College of Medicine, Houston, TX, USA
Presented By: Hunter Flores, BS

Oral-7

RBFOX2 COPY NUMBER VARIATION CAUSES HYPOSPADIAS BY DISRUPTING MESENCHYMAL TO EPITHELIAL TRANSITION IN PENIS DEVELOPMENT

Victor Ruthig[1], Fredi Ruiz Rojano[1,2], Marisol O'Neill[3], Jeffrey White[3], Dolores Lamb[1]
[1]Weill Cornell Medicine, New York, NY, USA, [2]New York Bioforce, New York, NY, USA, [3]Center for Reproductive Medicine, Houston, TX, USA
Presented By: Victor Ruthig, BA, MS, PhD

Oral-8

CAUSATIVE INFLUENCE OF TCTE1 KNOCKOUT ON ENERGETIC CHAIN TRANSPORTATION, APOPTOSIS AND SPERMATOGENESIS – IMPLICATIONS FOR MALE INFERTILITY

Marta Olszewska[1], Agnieszka Malcher[1], Tomasz Stokowy[2], Nijole Pollock[3], Andrea Berman[4], Sylwia Budkiewicz[1], Marzena Kamieniczna[1], Hanna Jackowiak[5], Piotr Jedrzejczak[6], Alexander Yatsenko[3], Maciej Kurpisz[1]
[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, [2]Department of Clinical Science, University of Bergen, Bergen, Norway, [3]Department of OB/GYN and Reproductive Sciences, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA, [4]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA, [5]Department of Histology and Embryology, Poznan University of Life Sciences, Poznan, Poland, [6]Division of Infertility and Reproductive Endocrinology, Department of Gynecology, Obstetrics and Gynecological Oncology, Poznan University of Medical Sciences, Poznan, Poland
Presented By: Marta Olszewska, MS, PhD

Oral-9

INVESTIGATION OF THE IMMUNE MILIEU OF THE EPIDIDYMIS: STRATEGIC POSITIONING OF LEUKOCYTES

Christiane Pleuger[1], Sudhanshu Bhushan[1], Daniel Bohnert[1], Minea Hoppe[1], Laura Winter[1], Kate Loveland[2], Mark Hedger[2], Andreas Meinhardt[1]

[1]*Justus-Liebig-University Giessen, Giessen, Germany,* [2]*Monash University, Clayton, Australia*
Presented By: Andreas Meinhardt, PhD

Oral-10

TARGETING THE MEIG1/PACRG INTERACTION FOR MALE CONTRACEPTION

Wei Li[1], Yi Tian Yap[1], Qian Huang[1,2], Ljiljana Mladenovic-Lucas[1], Shuo Yuan[1,2], Wei Qu[1,2], James Granneman[1], Zhibing Zhang[1]
[1]*Wayne State University, Detroit, MI, USA,* [2]*Wuhan University of Science and Technology, Wuhan, China*
Presented By: Wei Li, MD

Oral-11

HIGH-THROUGHPUT DRUG SCREENING METHOD FOR DISCOVERING NOVEL CATSPER INHIBITORS

Guillermina Luque[1], Martina Jabloñski[1], Liza Schiavi-Ehrenhaus[1], Paula Balestrini[1], Nicolas Torres[1], Dario Krapf[2], Mariano Buffone[1]
[1]*Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina,* [2]*Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Universidad Nacional de Rosario (UNR), Rosario, Argentina*
Presented By: Guillermina Luque, PhD

Poster 01

UNDERSTANDING THE ROLE OF UBIQUITIN LIGASE COMPLEX KCTD13-CUL3 IN HUMAN GENITOURINARY DEVELOPMENT

Abhishek Seth, Hunter Flores, Juan Bournat, Olga Medina-Martinez, Jill Rosenfeld, Liya Hu, Carolina Jorgez
Baylor College of Medicine, Houston, TX, USA
Presented By: Carolina Jorgez, PhD

Poster 02

LONG-TERM FOLLOW-UP AFTER HIGH-RISK GONADOTOXIC TREATMENT AND IMMATURE TESTICULAR TISSUE BANKING

Aude Braye[1], Inge Gies[1,2], Alina Ferster[3], Ellen Goossens[1]
[1]*Vrije Universiteit Brussel (VUB), Brussels, Belgium,* [2]*Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium,* [3]*Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), Brussels, Belgium*
Presented By: Aude Braye, MS

Poster 03

ENDOCRINE DISRUPTING CHEMICAL MIXTURES AFFECT THE INTERACTIONS BETWEEN MACROPHAGES AND SPERMATOGONIA

Haoyi Cui, Martine Culty
culty@usc.eduDepartment of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, CA, USA
Presented By: Haoyi Cui, MS

Poster 04

ROHYPNOL INDUCES MALE REPRODUCTIVE TOXICITY VIA SUPPRESSION OF CIRCULATORY ANDROGEN AND UPREGULATION OF CASPASE 3 SIGNALING

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Presented By: David Oluwole

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OMEGA 3 FATTY ACID BLUNTS TAMOXIFEN-INDUCED REPRODUCTIVE TOXICITY BY SUPPRESSING OXIDATIVE STRESS, INFLAMMATION AND APOPTOSIS

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Presented By: Moses Hamed

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ROLE OF BASAL CELL PRIMARY CILIA FROM THE EPIDIDYMIS IN THE CONTROL OF TISSUE REGENERATION AFTER INJURY

Laura Girardet[1], Daniel Cyr[2], Clémence Belleannée[1]
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Presented By: Laura Girardet

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SEGMENTAL DIFFERENTIATION OF THE MURINE EPIDIDYMIS: IDENTIFICATION OF SEGMENT-SPECIFIC, GM1-ENRICHED EXTRACELLULAR VESICLES AND REGULATION BY LUMINAL FLUID FACTORS

Danielle Sosnicki[1,2], Chinatsu Mukai[1], Atsushi Asano[3], Roy Cohen[1], Pierre Comizzoli[2], Alexander Travis[1]
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Presented By: Danielle Sosnicki, BS, MS

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CX3CR1 DEFICIENCY LEADS TO IMPAIRMENT OF THE IMMUNE SURVEILLANCE IN THE EPIDIDYMIS

Ferran Barrachina[1], Kiera Ottino[1], Leona Tu[1], Sylvie Breton[2], Maria Agustina Battistone[1]

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Presented By: Ferran Barrachina, BS, MS, PhD

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GENERATION OF NR5A1-INDUCIBLE HUMAN-LEYDIG LIKE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Lu Li, Chantal Sottas, Yuchang Li, Vassilios Papadopoulos
Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA, Los Angeles, CA, USA
Presented By: Lu Li

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A KNOCK-IN MOUSE MODEL FOR MALE FERTILITY: BASIS FOR THE MAMMAL-SPECIFIC PROTEIN PHOSPHATASE ISOFORM PP1Y2 IN SPERM

Wesam Nofal, Cameron Brothag, Mustfa Kabi, Huda Nofal, Srinivasan Vijayaraghavan
Kent State University, Kent, OH, USA
Presented By: Wesam Nofal, BS, MS

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Mahmoud Huleihel[1], Nagham Ali[1], Eitan Lunenfeld[2]
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Presented By: Mahmoud Huleihel, PhD

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Ryan Barney, Timothy Jenkins, Kelaney Stalker
Brigham Young University, Provo, UT, USA
Presented By: Ryan Barney, MS

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Jimmaline Hardy[1], Nijole Pollock[1], Chatchanan Doungkamchan[1], Jannah Kuong[1], Kien Tran[1], Yi Sheng[1], Thomas Jaffe[2], Marta Olszewska[3], Maciej Kurpisz[3], Kyle Orwig[1], Miguel Brieno-Enriquez[1], Alexander Yatsenko[1]
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Presented By: Jimmaline Hardy, MS, PhD

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Ana Romarowski[1], Saman Nayyab[2], David Hidalgo[3], Maria Gervasi[2], Ana Salicioni[2], Melanie Balbach[4], Lonny Levin[4], Jochen Buck[4], Jasna Fejzo[2], Pablo Visconti[2]
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Presented By: Ana Romarowski, PhD

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HIGH ANTI-SPERM ANTIBODY LEVELS ARE NOT ASSOCIATED WITH PREGNANCY OUTCOMES AFTER VASECTOMY REVERSAL

Catherine Nam[1], Ben Tooke[1], Mary Strasser[2], Mujtaba Hameed[1], Sadhana Chinnusamy[1], Stephanie Daignault-Newton[1], James Dupree[1]
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Presented By: Catherine Nam, MD

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Melanie Balbach, Thomas Rossetti, Lubna Ghanem, Carla Ritagliati, Peter Meinke, Lonny Levin, Jochen Buck
Weill Cornell Medical College, New York City, NY, USA
Presented By: Melanie Balbach, PhD

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THE ROLE OF CLOMIPHENE CITRATE IN OPTIMIZING SPERM COUNT, CONCENTRATION, AND MOTILITY IN INFERTILE MEN

Tristan Chun[1], Jacob Greenberg[1], Natalie Suder[1], Elliot Roufeh[2], Mallory Kiefer[1], Jacob Tannenbaum[1], Manesh Kumar Panner Selvam[1], Suresh Sikka[1], Omer Raheem[3], Wayne Hellstrom[1]
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Presented By: Tristan Chun, BS

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Tristan Chun[1], Jacob Greenberg[1], Natalie Suder[1], Elliot Roufeh[2], Mallory Kiefer[1], Christopher Koller[1], Jacob Tannenbaum[1], Manesh Kumar Panner Selvam[1], Suresh Sikka[1], Omer Raheem[3], Wayne Hellstrom[1]
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Presented By: Tristan Chun, BS

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CLOMIPHENE CITRATE TREATMENT RESPONSE OF SEMEN PARAMETERS AND TESTOSTERONE LEVELS IN HYPOGONADAL MEN OVER TIME

Michael Basin, Connor Policastro, Anastasija Useva, Carla Miguel, JC Trussell
SUNY Upstate Medical University, Syracuse, NY, USA
Presented By: Michael Basin, MD

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USE OF MELATONIN AND CAFFEINE IN CRYOPRESERVATION PROCESS BY SLOW-FREEZING METHOD INCREASES POST-THAW SPERM MOTILITY

Juliana Pariz[1,2], Larissa Chiba[2], Mayara Rodrigues[1,2], Heloisa Faquineti[2,3], Ana Clara Barduchi[2], Mabel Schulz[4], Joel Drevet[5], Raul Sanchez[4], Jorge Hallak[2,3]
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Presented By: Juliana Pariz, PhD

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ASSESSMENT OF GENOTOXICITY OF VAS-OCCLUSIVE MALE CONTRACEPTIVE ON SPERM

Kevin Eisenfrats, Rucha Bhat
Contraline, Inc., Charlottesville, VA, USA
Presented By: Kevin Eisenfrats

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PRE-PUBERTAL CODEINE EXPOSURE EXAGGERATES MATERNAL CODEINE EXPOSURE-INDUCED DELAY IN PUBERTY ONSET AND SEXUAL DYSFUNCTION IN MALE WISTAR RAT: POSSIBLE ROLE OF ARGININE

Roland Akhigbe, Oladele Afolabi, Ayodeji Ajayi
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Presented By: Roland Akhigbe, MBBS

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SLEEP DISORDERS ARE ASSOCIATED WITH HYPOGONADISM AND ERECTILE DYSFUNCTION - A U.S. CLAIMS DATABASE ANALYSIS

Pranjal Agrawal[1], Sajya Singh[1], Corey Able[2], Taylor Kohn[1], Amin Herati[1]
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Presented By: Pranjal Agrawal

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Oleksandr Knigavko[1], Yurii Avdosiev[2], Iryna Fesenko[1]
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Presented By: Oleksandr Knigavko, MD, PhD

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Moshe Wald, Fiona Armstrong-pavlik
University of Iowa, Iowa City, IA, USA
Presented By: Moshe Wald, MD

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Abinav Udaiyar[1], Robert Wilson[2], Stanley Kogan[1,3], David Childs[4], Marc Colaco[3], Marshall Schwartz[1,3], Steve Hodges[1,3], Anthony Atala[1,3], Hooman Sadri-Ardekani[1,3]
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Presented By: Abinav Udaiyar, BS

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Alexander Hauser, Ann Kiessling, Mark Hartley, Maureen Kearnan

Bedford Research Foundation, Bedford, MA, USA
Presented By: Alexander Hauser, BS

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Gayatri Mohanty, Maria Gervasi, Darya Tourzani, Pablo Visconti
Department of Veterinary and Animal Sciences, Integrated Sciences Building, University of Massachusetts, Amherst, MA, USA
Presented By: Gayatri Mohanty, PhD

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MALE FERTILE POTENTIAL ASSESSMENT AMONG HIGH AND LOW-INTENSITY CYCLISTS

Luana Adami, Valter Maciel Junior, Ricardo Bertolla
University Federal of Sao Paulo, Sao Paulo, Brazil
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Larissa Chiba[1,2], Juliana Pariz[3], Heloisa Faquineti[1], Raul Sanchez[4], Joel Drevet[5], Mabel Andrea Rubilar Schulz[4], Jorge Hallak[1,2,6,7]
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Presented By: Larissa Chiba, BS

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EJACULATED HUMAN SPERM CYCLE BETWEEN STATES OF FERTILITY AND INFERTILITY, THE KNOWLEDGE OF WHICH CAN PREDICT IUI SUCCESS

Clifford Librach[1], Sergey Moskovtsev[1], Peter Wieckowski[2], Junyan Zhang[1], Kimberly Bingham[2], Amy Coffey[2], John Walsh[3], Zimu Chen[3], Paul Turek[4], Erin Schnellinger[5], Ronald Parkinson[3], Barb Cohen[3], Joseph Hill[2]
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Presented By: Clifford Librach, MD

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META-ANALYSIS REVEALS ASSOCIATION BETWEEN TWO SPERM FUNCTION ASSAYS: SEMINAL OXIDATION REDUCTION POTENTIAL AND SPERM DNA FRAGMENTATION

Manesh Kumar Panner Selvam[1], Saradha Baskaran[1], Samantha O'Connell[2], Wael Almajed[1], Wayne Hellstrom[1], Suresh Sikka[1]
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Presented By: Manesh Kumar Panner Selvam, DVM, PhD

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EXPANDING ACCESS TO MALE FERTILITY TESTING THROUGH VALIDATION OF AN AT HOME COLLECTION KIT

G Charles Ostermeier[1], Cristina Cardona[1], Alexander Travis[1,2]
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Presented By: G Charles Ostermeier, PhD

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IN MEN SEEKING FERTILITY ASSISTANCE, DEFECTS IN SPERM CAPACITATION/FERTILIZING ABILITY ARE COMMON IN ALL AGE GROUPS, IN CONTRAST, SEMEN VOLUME AND MOTILITY DECLINED WITH AGE

Fady Sharara[1], Cristina Cardona[2], Charles Ostermeier[2], Alexander Travis[2,3]
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Presented By: Cristina Cardona, PhD

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Anastasiya Spaska[1], Bogdan Grytsuliak[2]
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Presented By: Anastasiya Spaska, PhD

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MOUSE ADAPTED SARS-COV-2 INDUCES HISTOPATHOLOGICAL CHANGES IN TESTIS OF LABORATORY MICE

Manesh Kumar Panner Selvam[1], Akhilesh Srivastava[1], Felix Rabito[1], Amruta Narayanappa[1], Gregory Bix[1], Xuebin Qin[2], Jay Kolls[1], Suresh Sikka[1]
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Presented By: Manesh Kumar Panner Selvam, DVM, PhD

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Yi Tian Yap[1], Shuo Yuan[1,2], Wei Li[1], Wei Qu[1,2], Ljiljana Mladenovic-Lucas[1], Alyson Sujkowski[1], Robert Wessells[1], Jie Xu[3], Rex Hess[4], James Granneman[1], Aminata Toure[5], Jifeng Zhang[3], Zhibing Zhang[1]
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Presented By: Yi Tian Yap, BS

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CHARACTERIZATION OF TSPAN33 AND LPPR3 HUMAN SPERMATOGONIAL STEM CELLS

Andre Caldeira-Brant[1], Sarah Munyoki[1], Meena Sukhwani[1], Helio Chiarini-Garcia[2], Kyle Orwig[1]
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Presented By: Andre Caldeira-Brant, PhD

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Penny Whiley[1,2], Michael Luu[1], Robin Hobbs[1], Kate Loveland[1,2]
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Presented By: Penny Whiley

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RELIABILITY AND QUALITY OF NEOVAGINOPLASTY CONTENT ACROSS YOUTUBE AND TIKTOK

Richard Mateo Mora, Jack Rodman, Mary Samplaski
University of Southern California Institute of Urology, Los Angeles, CA, USA
Presented By: Richard Mateo Mora, BS

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SYSTEMATIC REVIEW: THE NEOVAGINAL MICROBIOME

Richard Mateo Mora[1], Preeya Mehta[2], Ryan Ziltzer[2], Mary Samplaski[1]
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Presented By: Richard Mateo Mora, BS

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CHARACTERISTICS OF SYSTEMIC ANDROGEN THERAPY FOR HYPOACTIVE SEXUAL DESIRE DISORDER IN FEMALES - A CLAIMS DATABASE ANALYSIS

Pranjal Agrawal, Kathryn Dumas, Taylor Kohn, Jaden Kohn, Marisa Clifton
Johns Hopkins University School of Medicine, Baltimore, MD, USA
Presented By: Pranjal Agrawal, BA

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CACHEXIA MANAGEMENT WITH TESTOSTERONE REPLACEMENT THERAPY: A CONTEMPORARY REVIEW OF THE LITERATURE

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Presented By: Armaan Singh, BA

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EXOGENOUS LEPTIN TREATMENT ALTERS THE TESTICULAR IMMUNE MICROIMMUNE-ENVIRONMENT

Alexandra Dullea, Deepa Seetharam, Kajal Khodamoradi, Isabelle Issa, Natoli Farber, Ranjith Ramasamy, Himanshu Arora
University of Miami, Miami, FL, USA
Presented By: Alexandra Dullea, MS

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TAMOXIFEN ALTERS TESTICULAR STEROIDOGENESIS AND SPERMATOGENESIS VIA AN OXIDATIVE STRESS-MEDIATED SIGNALING: POSSIBLE ROLE OF CESSATION

Roland Akhigbe[1], Adeyemi Odetayo[2], Moses Hamed[3], David Oluwole[1]
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Presented By: Roland Akhigbe, MBBS

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EFFECTS OF ENDOCRINE DISRUPTING CHEMICALS, ACETAMINOPHEN, AND NSAIDS ON IMMATURE SERTOLI CELL FUNCTIONS

Maia Corpuz, Amy Tran, Martine Culty
University of Southern California, Los Angeles, CA, USA
Presented By: Maia Corpuz, BS, MS

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Larissa Berloffa Belardin[1], Dana Sandor[2], Diane Capen[2], Dennis Brown[2], Christine Légaré[1], Sylvie Breton[1]
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Presented By: Larissa Berloffa Belardin, PhD

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CHARACTERIZATION OF ATP RELEASE BY PRINCIPAL CELLS IN THE EPIDIDYMIS

Kéliane Brochu[1,2], Christine Légaré[1,2], Sylvie Breton[1,2]
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Presented By: Kéliane Brochu, BS

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NOVEL MECHANISMS OF HOST DEFENSE IN THE EPIDIDYMIS

Caitlyn Myers[1], Mary Catherine Hastert[2], Gail Cornwall[1]
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Presented By: Caitlyn Myers, BS

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"SPERM EXTRAGENOMIC CONTRIBUTIONS IN PREGNANCY LOSS AND CONGENITAL MALFORMATIONS: A CASE CONTROL STUDY"

Vidhu Dhawan, Manoj Kumar, Neena Malhotra, Neeta Singh, Vatsla Dadhwal, Rima Dada
All India Institute of Medical Sciences, New Delhi, India, New Delhi, India
Presented By: Vidhu Dhawan, MBBS, MD

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SPERM INCUBATION CONDITIONS INFLUENCE THE SUCCESS OF PRE-IMPLANTATION EMBRYO DEVELOPMENT

Darya Tourzani[1], Erica Jackson[1], Maria Gervasi[1,2], Pablo Visconti[1]
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Presented By: Darya Tourzani, BS

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DEVELOPMENT OF A NOVEL METHOD FOR ASSESSING CLINICAL EFFICACY OF AN ON-DEMAND MALE CONTRACEPTIVE

Carla Ritagliati, Melanie Balbach, Lonny Levin, Jochen Buck
Weill Cornell Medicine, New York, NY, USA
Presented By: Carla Ritagliati, BS, PhD

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Yanhe Lue[1], Luyang Yu[2], Junze Liu[2], Hang Yang[2], Hyle Park[2], WonHo Kim[1], Youngju Pak[1], Jacob Rajfer[3], Christina Wang[1], Ronald Swerdloff[1]
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Presented By: Yanhe Lue, MD

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SPERMCHECK FOR USER-MONITORING OF SPERM SUPPRESSION IN A HORMONAL MALE CONTRACEPTIVE TRIAL

Yanhe Lue[1], Ronald Swerdloff[1], Youngju Pak[1,2], Fiona Yuen[1], Peter Liu[1], Brian Nguyen[3], Diana Blithe[4], Christina Wang[1,2]
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Presented By: Yanhe Lue, MD

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COMPARATIVE ACCURACY OF THE NOVEL SPERMAN® SPERM SELECTION TECHNOLOGY VERSUS SWIM UP METHOD

Aysun Fiori, Burak Özkösem
Pera Labs, Philadelphia, PA, USA
Presented By: Burak Özkösem, PhD

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DEVELOPMENT OF A NOVEL CONTRACEPTIVE FOR MEN

Nadja Mannowetz[1], Gunda Georg[2], Debra Wolgemuth[3], Henry Wong[2], Akash Bakshi[1]
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Presented By: Nadja Mannowetz, PhD

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NOVEL CONTRACEPTIVE METHOD: BLOCKING OF SERINE PROTEASE ACTIVITY TO PREVENT SEMEN LIQUEFACTION

Prashanth Anamthathmakula[1,2], Wipawee Winuthayanon[1]
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Presented By: Wipawee Winuthayanon, BSN, PhD

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IMPACT OF YOGA ON UNEXPLAINED INFERTILITY, COMORBID DEPRESSION AND QUALITY OF LIFE

Rajesh Kumar[1], Manoj Kumar[1], Rajeev Kumar[1], Taruna Katyal[2], Rima Dada[1]
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Presented By: Rajesh Kumar, MBBS, MD

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THE EFFECT OF LEPROSY ON MALE FERTILITY AND SEXUAL FUNCTION

Richard Mateo Mora[1], Jack Rodman[1], Maria Ochoa[2], Mary Samplaski[1]
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Presented By: Richard Mateo Mora, BS

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COVID-19 AND MALE REPRODUCTION: A SCIENTOMETRIC STUDY

Manesh Kumar Panner Selvam[1], Anika Kapoor[2], Saradha Baskaran[1], Suresh Sikka[1]
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Presented By: Manesh Kumar Panner Selvam, PhD

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LIVE SPERMATOZOA MORPHOMETRY BY DIGITAL HOLOGRAPHIC MICROSCOPE

Marzena Kamieniczna[1], Agata Augustynowicz[2], Ewa Stachowska[2], Maciej Kurpisz[1]
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fahim rajiwate, dr vasan ss, ashish reddy
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ABSTRACTS

Oral-1

IN VITRO SPERMATOGENESIS FROM IMMATURE TESTICULAR TISSUES CRYOPRESERVED FOR PEDIATRIC PATIENTS BEFORE GONADOTOXIC THERAPY: A STEP TOWARDS FERTILITY PRESERVATION AND RESTORATION

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Presented By: Nagham Younis, MS

Introduction & Objective: Chemotherapy and radiation treatments can cause permanent infertility. Cryopreservation of immature testicular tissue (ITT) is the only fertility preservation option for pediatric patients who are not producing sperm. Those tissues or cells can be transplanted back into the patients in the future to restore sperm production and/or fertility. However, autologous transplantation may not be appropriate or safe for all patients (e.g., leukemia patients). Therefore, methods are needed to mature testicular tissue and produce sperm outside the patient's body. Activation of spermatogenesis from ITT has been achieved in mice, but has not been replicated in any other species. The objective of this study is to induce in vitro spermatogenesis from pediatric patient ITT that was cryopreserved prior to treatment.

Methods: An organotypic culture system was used to study in vitro spermatogenesis from ITT of prepubertal and peripubertal pediatric patients. Tissues were cultured for 7, 16, and 32 days in medium with no supplements or supplemented with FSH, HCG or FSH + HCG. Tissues were evaluated by histology and immunofluorescence to assess tissue integrity as well as the presence of VASA+ germ cells, PGP9.5+ spermatogonia, SYCP3+ spermatocytes, CREM+ spermatids, SOX9+ Sertoli cells, and INSL3+ Leydig cells. We also assessed proliferation and apoptosis of germ cells and Sertoli cells and testosterone levels in the culture medium.

Results: Hematoxylin and eosin-stained sections revealed that the integrity of seminiferous tubules was maintained throughout the culture period. VASA+ germ cells were observed in all cultured ITT fragments at all time points and all treatment groups. PGP9.5+ Spermatogonia and SOX9+ Sertoli cells were observed in all samples. SYCP3+ spermatocytes began to appear in some samples from days 16 and 32 in culture. Assessments of CREM+ spermatids and INSL3+ Leydig cells are ongoing but the presence of functional Leydig cells is indicated by the secretion of testosterone into the culture medium. Finally, we are in the process of quantifying germ cell/somatic cell proliferation (Ki67) and apoptosis (Caspase 3).

Conclusion: Immature testicular tissues could be maintained in culture for up to 32 days with germ cell survival and sporadic differentiation to produce meiotic and post-meiotic cells.

Oral-2

SPERM MRNA PROFILES REFLECT REPRODUCTIVE HISTORY IN PATIENTS PRESENTING FOR VASECTOMY OR INFERTILITY ASSESSMENT

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Presented By: Daniel Spade, PhD

Introduction & Objective: Idiopathic male infertility is very common, representing approximately half of male infertility cases. Analysis of idiopathic infertility is limited to semen analysis, which is moderately predictive of ability to conceive either through intercourse or assisted reproductive technology. Spermatozoa carry thousands of RNAs. In addition to regulatory small RNAs, over 10,000 mRNA transcripts have been sequenced in purified human spermatozoa. These mRNAs are largely derived from the germ cells prior to transcriptional silencing, and may serve as a record of the biological states of germ cells during spermatogenesis. We have previously shown that the sperm mRNA profiles of humans, rats, and mice are very similar, suggesting that they reflect conserved spermatogenesis-related functions. We have also shown that lifestyle factors influence the total amount of RNA in human spermatozoa and that rat sperm mRNAs can be used as indicators of testicular toxicity. In the present study, we hypothesized that sperm mRNA profiling could distinguish idiopathic infertile men from known fertile men.

Methods: We isolated sperm mRNA from two patient populations at Brown Urology: patients who presented for infertility assessment (n = 56, "infertility"), and patients who had fathered a child within the past three years and presented for vasectomy (n = 14, "pre-vasectomy"/known fertile control). Samples were excluded from analysis if any semen parameter was below the WHO reference value. RNA libraries were prepared for sequencing using poly-A selection, and libraries were sequenced on the Illumina HiSeq in paired-end, 150 bp configuration to an average read depth of 30,000 reads/sample.

Results: We found that there was surprisingly strong segregation between infertility and pre-vasectomy samples in unsupervised analyses, including hierarchical clustering and principle components analysis. One thousand six hundred seventy-eight total transcripts were significantly differentially abundant between the patient populations

($q < 0.05$, fold difference≥2), with 63.8% of significant transcripts downregulated in infertility patients. In functional enrichment analysis, the Gene Ontology term, Spermatogenesis, was significantly enriched. Twenty-eight of 30 significant genes under this term, including PRM1, PRM2, PRM3, TNP2, KIT, and BOLL, were significantly downregulated in infertility patients.

Conclusion: These results provide evidence that sperm mRNA populations differ between men with different reproductive histories and support the hypothesis that spermatogenesis-associated transcripts could act as markers of male fertility or provide mechanistic information about spermatogenic deficiencies in idiopathic infertile men.

Oral-3

TRANSCRIPTOMIC DIFFERENCES BETWEEN FIBROTIC AND NON-FIBROTIC TESTICULAR TISSUE REVEAL POSSIBLE KEY PLAYERS IN KLINEFELTER SYNDROME-RELATED TESTICULAR FIBROSIS

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Presented By: Margo Willems, BS, MS

Introduction & Objective: Klinefelter syndrome (KS; 47,XXY) affects 1-2 in 1000 males. Most (95%) KS men suffer from azoospermia due to the loss of spermatogonial stem cells. Additionally, testicular fibrosis is detected from puberty onwards. However, mechanisms responsible for fibrosis and germ cell loss remain unknown. This study aimed to identify factors which may be involved in the fibrotic remodeling of KS testes by analyzing the transcriptome of (non-)fibrotic testicular tissue.

Methods: RNA sequencing was performed to compare the genetic profile of testicular biopsies from patients with (KS and testis atrophy) and without (Sertoli cell-only syndrome and fertile controls) testicular fibrosis ($n = 5$, each). In addition, differentially expressed genes (DEGs) between the KS and testis atrophy samples were studied to reveal KS-specific fibrotic genes. DEGs were considered significant when $p < 0.01$ and $\log2FC > 2$. To gain insight in the potential functions of the DEGs, gene-ontology and KEGG analyses were performed. To validate the gene expression results, immunohistochemistry and RNA scope were performed.

Results: Transcriptomic analysis of fibrotic versus non-fibrotic testis tissue resulted in 734 significant DEGs (167 up- and 567 downregulated), of which 26 were X-linked. In the top upregulated biological functions, DEGs involved in the extracellular structure organization were found, including vascular cell adhesion molecule 1 (VCAM1). KEGG analysis showed an upregulation of genes involved in the TGF-β pathway. The second analysis of KS versus testis atrophy samples resulted in 539 significant DEGs (59 up- and 480 downregulated). One of the biological functions found though gene-ontology analysis was the chronic inflammatory response. When looking at the overlap of DEGs on the X-chromosome from the first analysis, three genes were found: matrix-remodeling associated 5 (MXRA5), doublecortin (DCX) and variable charge X-Linked 3B (VCX3B). As validation, an overexpression of VCAM1, MXRA5 and DCX was found within the fibrotic group compared to the non-fibrotic group through immunohistochemistry and RNA scope.

Conclusion: Comparing DEGs between fibrotic and non-fibrotic tissue resulted in genes which may play a role in testicular fibrosis, including VCAM1. When comparing the X-linked genes of the first analysis with those of the second analysis (KS versus testis atrophy) were compared, fibrotic genes on the X-chromosome were revealed. MXRA5, DCX and VCX3B may play a role in KS-related testicular fibrosis.

Oral-4

DECODING THE MOLECULAR DEFICIENCIES UNDERLYING OLIGOASTHENOTERATOSPERMIA: IDENTIFICATION OF AARDC5 DEFICIENCY

Mariana Ianello Giassetti, Deqiang Miao, Melissa Oatley, Nate Law, Jon Oatley

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Presented By: Mariana Ianello Giassetti, DVM, PhD

Introduction & Objective: The most common form of male factor infertility is oligoasthenoteratospermia (OAT) which describes an ejaculate with low sperm count, poor sperm motility, and increased abnormal sperm morphology. At present, mammalian models of OAT are limited and specific molecular deficiencies that lead to OAT are largely undefined.

Methods: In this study, we conducted a multispecies (mice, cattle, pigs, and humans) comparative single cell RNA sequencing (scRNA-seq) analysis of testicular tissue to identify novel germ cell specific gene expression that is evolutionarily conserved across mammalian.

Results: Bioinformatics analysis revealed 241 genes differentially expressed in germ cells of all the species including the E3 ubiquitin ligase Arrdc5. In alignment with the GTEX database of gene expression in human tissues, assessment of Arrdc5 expression in several tissues of adult mice by RT-PCR indicated testis specificity. Using CRISPR-Cas9 technology, we created an Arrdc5 knockout mouse line. Based on mating trials, Arrd5-/- male mice were found to be sterile, whereas knockout females were fertile. Assessment of epididymal sperm revealed that knockout males have oligoasthenoteratospermia with significant reductions in epididymal sperm concentration and motility, as well as severe increase in abnormal morphology including macrospermia. Sperm from Arrdc5-/- males also possessed malformed acrosomes, abnormal nuclear condensation, impaired mitochondrial sheath arrangements, and disrupted flagellar assembly. Consequently, sperm from Arrdc5-/- males were unable to undergo normal capacitation and filed to bind oocytes during in vitro fertilization conditions

Conclusion: Collectively, these findings uncover ARRDC5 as a testis specific molecule that plays a critical role in regulating

spermatogenesis and conferring male fertility. Moreover, the Arrdc5 knockout mouse is a model of OAT that could be used to further our understanding of the molecular basis for this infertility condition in men

Oral-5

MUTATIONS AND MICRODELETIONS IN PRSS50 AND LRWD1 AFFECT MALE FERTILITY

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Presented By: Hunter Flores, BS

Introduction & Objective: Despite advances in molecular diagnostics, the etiology of most male infertility remains unknown or undiagnosed. Multiple morphological abnormalities of the sperm flagella (MMAF) is a major cause of asthenoteratozoospermia. We identified the protease, PRSS50, with a crucial role in sperm development since *Prss50*-null mice present with impaired fertility and sperm tail abnormalities. PRSS50 could play a role in centrosome and tail formation since *Prss50*-null mice present with a three-fold increase in acephalic sperm, sperm with multiple heads, and spermatozoa with multiple tails including the novel two-sperm-conjoined. We identified LRWD1 as one of the genes regulated by PRSS50. LRWD1 is highly enriched in the cytoplasm of spermatocytes and in the centrosome. Three synonymous single-nucleotide variants (SNV) in LRWD1 have been associated with Sertoli Cell Only, but no PRSS50 SNV has been associated with infertility. Our objective is to determine if copy number variants (CNVs) and SNV in PRSS50 and LRWD1 are associated with infertility.

Methods: We generated a *Lrwd1*-KO mice to study fertility. We also selected 6 male patients with known morphological abnormalities in spermatozoa, and performed Sanger sequencing in all axons of LRWD1 and PRSS50. We also performed CNV assays of PRSS50 in a cohort of 241 infertile men.

Results: Although *Lrwd1*-KO mice seem to be fertile, 70% of their sperm are acephalic, indicating the importance of *Lrwd1* in neck formation as a centromere protein. We also identified a 58Kb microdeletion at chromosome 3p21.31 encompassing in the PRSS cluster in four of 20 NOA men absent in 20 controls. Validation and analysis by qPCR of a total of 241 NOA men identified 32 NOA men with microdeletion (nine of them have 0 copy of the gene) and seven with microduplication (three copies). From the 241 controls, 21 of them have microdeletions lacking only one copy. The absence of both copies or extra copy of the gene was significant difference when compared to control ($p = 0.01$). Sanger sequencing of PRSS50 identified two possible damaging non-synonymous SNVs in exon-2 (Q75P) and exon-4 (R239C). In LRWD1, we found a previously identified synonymous SNV in exon-13 in all the patients.

Conclusion: Gene-dosage changes of 3p21.31 encompasses the PRSS cluster, and PRSS50 mutations represent a previously unrecognized cause of infertility. This study demonstrates that PRSS50 and LRWD1 play a role in MMAF syndrome.

Oral-7

RBFOX2 COPY NUMBER VARIATION CAUSES HYPOSPADIAS BY DISRUPTING MESENCHYMAL TO EPITHELIAL TRANSITION IN PENIS DEVELOPMENT

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Presented By: Victor Ruthig, BA, MS, PhD

Introduction & Objective: Despite hypospadias frequency (1:125 live male births) the etiology is often not known. The Lamb Laboratory previously determined that RNA Binding Fox-1 Homolog 2 (*RBFOX2*) copy number variation (CNV) is associated with clinical cases of upper and lower tract genitourinary anomalies, including hypospadias. *RBFOX2* encodes an RNA-binding protein that regulates mRNA splicing. The hypothesis was tested that altered *RBFOX2* gene dosage impacts the developing penis transcriptome by disrupting mRNA splicing. Specifically vitiating the mesenchymal to epithelial transition critical for penile urethra formation, resulting in hypospadias.

Methods: Immunofluorescenet and in situ hybridization imaging along a fetal time course of penis development defined anatomical regions of *Rbfox2* transcript and protein expression. To identify developmental pathways regulated by RBFOX2 in the genitourinary system, *Rbfox2*-KO (null) mice were developed to model the clinical phenotype. Penises were collected for RNA-seq analysis. Differential analysis comparing P1 penises of *Rbfox2*-KO and wild type siblings defined the transcriptome and splice-ome changes caused by *Rbfox2* loss. Significant differentially used genes were anatomically mapped in the developing fetal penis (E14.5-E16.5) using published scRNA-seq datasets [Amato & Yao 2021, Armfield & Cohn 2021]. RNA immunoprecipitation (RIP) was used to distinguish between direct RBFOX2 transcript targets and downstream affected genes.

Results: Imaging analysis on fetal mouse penises revealed, after penile sex determination (E15.5), *Rbfox2* expression becomes restricted to the urethral mesenchyme and distal glans. Differential analysis on the *Rbfox2*-KO mouse penis transcriptome and splice-ome identified changes associated with *Rbfox2* loss in epithelial differentiation, pluripotency, keritizaton, hormone, and Tgf-beta signalling. Annotation of these affected genes, to regions of the developing penis using scRNA-seq data, localized some genes as regionally restricted to penis epithelium or mesenchyme. RIP agianst RBFOX2 in human kidney cells validated mRNA transcipt targets previously identified in cardiac cells [Verma 2016].

Conclusion: *RBFOX2* gene dosage alterations, clinically correlate with upper and lower tract genitourinary anomalies, specifically hypospadias. Data from human cell lines and mouse models seemingly indicate the phenotype is partially the product RBFOX2 directing the mesenchymal to epithelial transition. In addition to directing this transition there may be mechanistic differences between RBFOX2 action in epithlial versus mesenchymal regions, RBFOX2 is a bi-modal RNA

binding protein. These findings have implications for other regions of the genitourinary tract such as gonad and kidney.

Oral-8

CAUSATIVE INFLUENCE OF TCTE1 KNOCKOUT ON ENERGETIC CHAIN TRANSPORTATION, APOPTOSIS AND SPERMATOGENESIS – IMPLICATIONS FOR MALE INFERTILITY

Marta Olszewska[1], Agnieszka Malcher[1], Tomasz Stokowy[2], Nijole Pollock[3], Andrea Berman[4], Sylwia Budkiewicz[1], Marzena Kamieniczna[1], Hanna Jackowiak[5], Piotr Jedrzejczak[6], Alexander Yatsenko[3], Maciej Kurpisz[1]

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Presented By: Marta Olszewska, MS, PhD

Introduction & Objective: One of the nexin-dynein regulatory complex structural gene is *TCTE1* (T-Complex-associated-Testis-Expressed 1; MIM:186975/MGI:98640); evolutionary conserved in eukaryotic organisms and required for proper flagella functioning. We aimed to determine the *Tcte1* role in male infertility using mouse knockout model. We created homo- and heterozygous animals, checked their reproductive potential, differences in anatomy/histology, and evaluated semen parameters. We performed RNA sequencing to check the influence of *Tcte1*$^{-/-}$ mutation on the other gonadal genes' expression. ATP measurement of spermatozoa was performed. Additionally, screening of human variants from males with drastically decreased sperm count, followed by protein prediction modeling was performed.

Methods: Creation of mouse knockout line of *Tcte1*: CRISPR/Cas9 on the basis of C57Bl/6J strain (exon-3 deleted). Number of litters, pups, sex ratio were estimated (genotyping via PCR). Gross anatomy and classic histological evaluation on testis and epididymis. RNAseq: Illumina NovaSeq 6000 platform, TruSeq Stranded-mRNA-LT-Sample-Prep-Kit. Protein-protein interactions (STRING): for genes with changed expression level in testis. Immunofluorescence in situ: detection of the N-DRC proteins: Tcte1, Drc7, Fbxl13, Eps8l1. Measurement of ATP: CellTiter-Glo 2.0 Assay. Homology modeling: Phyre2 and I-TASSER. Sequencing: WGS, WES, Sanger on n = 248 human samples from infertile males (azoo-/crypto-/severe oligozoospermia).

Results: We observed two phenotypes dependent on zygosity: homozygous males manifested oligoasthenoteratozoospermia (OAT; decreased testis size) and were infertile, while heterozygous males demonstrated oligozoospermia (O) and remained fertile. Observed circular movement pathway of spermatozoa, and increased ratio of immotile sperm cells in *Tcte*$^{-/-}$, resulted from inflexibility of sperm midpiece according to changed protein charge level in Tcte1, elevated lengths of sperm tail and midpiece, and decreased amount of ATP produced. *Tcte1* mutations influenced the expression pattern of 21 genes related to: mitochondrial cytochrome oxidase activity (diminished), apoptosis (increased), and spermatogenesis (diminished). Protein prediction modeling of identified heterozygous variants (one novel, three ultrarare, two rare; causative damaging effect) in humans, revealed changes/deformation in the protein surface, suggesting the disrupted TCTE1 interaction with its axoneme binding structures.

Conclusion: *TCTE1* gene plays crucial role in spermatogenesis and proper sperm functioning. Mouse knockout model revealed two phenotypes, dependent on zygosity, underlining the wide influence on male fertility. Novel functions and networks between differentially expressed genes were established, especially linking energy transportation, apoptosis and spermatogenesis, indicating sophisticated *Tcte1* role in reproduction.

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Oral-9

INVESTIGATION OF THE IMMUNE MILIEU OF THE EPIDIDYMIS: STRATEGIC POSITIONING OF LEUKOCYTES

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Presented By: Andreas Meinhardt, PhD

Introduction & Objective: The murine epididymis consists of a single duct meandering through four distinct regions (initial segment, caput, corpus, cauda) that face opposing immunological challenges. In the proximal regions, local tolerance is required to avoid autoimmune reactions against immunogenic spermatozoa. Conversely, the cauda represents the entry site for bacterial pathogens ascending from the urethra. Generally, the cauda is more susceptible to severe immune responses following bacterial infection, an observation that is frequently associated with male infertility in mice and men. It is hypothesized that immune cell populations are strategically positioned along the epididymis to maintain the immunological equilibrium across the organ.

Methods: Murine epididymitis was elicited by luminal injection of uropathogenic *Escherichia coli* (UPEC) in the vas deferens. Morphological alterations were assessed by histological staining and related to quantity and localization of infiltrating leukocyte populations by flow cytometry and immunofluorescence. Epididymal regions from wt mice were simultaneously exposed to an inflammatory stimulus ex vivo (LPS, 50 ng/mL, 6 h). Levels of inflammatory cytokines (e.g. TNFα, MCP-1, IL-1α, IL-1β, IL-6) were quantified using RT-qPCR and bead-based immunoassay. Multipanel flow cytometry and immunofluorescence

using CX3CR1^{GFP}CCR2^{RFP} mice were performed to characterize resident immune cells under physiological conditions.

Results: UPEC infection resulted in severe immune responses within the cauda associated with epithelial detachment and fibrotic remodeling, while the caput remained mostly unresponsive. Tissue-damage was positively correlated with an increase of CD45^{+} leukocytes, i.e. infiltrating Ly6G^{+} neutrophils followed by Ly6C^{hi}CD11b^{hi}F4/80^{lo} monocytes that appear to differentiate into Ly6C$^{lo\text{-}int}$CD11b^{hi}F4/80^{int}MHC-II^{hi} macrophages. Immune cell infiltration was associated with upregulation of inflammatory cytokines within the cauda in ascending bacterial infection in vivo, similar to the ex vivo model where LPS challenge occurred simultaneously.

At steady-state, immune cell populations exhibited striking region-specific properties. The proximal regions were densely populated by immunoregulatory immune cells, i.e. macrophage subsets involved in tissue homeostasis (identified by alternating expression of CX3CR1, CCR2, MHC-II, CD206). Corpus and cauda were characterized by a smaller but more diverse network of immune cells (T cells, NK cells, DC subsets, CCR2^{+} monocyte-derived macrophages) suggesting an immune-sensitive environment within the distal regions predestined to sense invading pathogens.

Conclusion: Our data suggest that strategically positioned leukocyte populations are key in maintaining the immunological equilibrium within the epididymis and thus could be responsible for the differences in immune responsivenesses of epididymal regions.

Oral-10

TARGETING THE MEIG1/PACRG INTERACTION FOR MALE CONTRACEPTION

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Presented By: Wei Li, MD

Introduction & Objective: Development of male contraceptives has lagged far behind that of female contraceptives. Current physical options for male birth control have limitations with respect to reliability, consistency of use, and invasiveness, respectively. Inhibiting sperm production by decreasing testosterone levels would also cause unacceptable side effects. Thus, developing a contraceptive that blocks the late stage of spermatogenesis without the use of hormones so that the effect is reversible with fewer/no side effects. The manchette is a transient microtubule-containing structure that is present only in elongating spermatids, and genetic disruption of the manchette structure/function results in male infertility. Importantly, we have discovered that the function of the machete in spermatogenesis requires the interaction between meiosis expressed gene 1 (MEIG1) and Parkin co-regulated gene (PACRG). A single point mutation in MEIG1 or PACRG that disrupts the interaction of the two proteins also disrupts spermatogenesis resulting in pure male infertility. Interaction between MEIG1 and PACRG is conserved in humans. We hypothesize that compounds that disrupt the MEIG1/PACRG interaction can be developed into safer and effective male contraceptives.

Methods: Availability of the MEIG1/PACRG structure makes it possible to in silico virtual and artificial intelligence (AI) screens for small molecules that block MEIG1/PACRG interaction, with biochemical validation hits. In addition, a fragment library for compounds is available for a physical screen and the fragments that interrupt MEIG1/PACRG interaction can also be used for drug development in the future.

Results: To this end, we developed a robust G. princeps luciferase complementation assay (PCA) using cell lysates and a more sensitive and stable NanoLuc® Binary Technology (NanoBiT®) assay on live cells for the interaction of MEIG1 and PACRG that can be readily used to validate the effect of the small molecules identified from the virtual and AI screens to interrupt MEIG1/PACRG interaction.

Conclusion: Given that global Meig1 knockout mice and both single amino acid mutant MEIG1 or PACRG mice showed a male infertility only phenotype, targeting MEIG1/PACRG interaction is believed to cause few/no side effects. The ultimate goal is to advance a male contraceptive to inhibit sperm formation/function. The discovery and development of a male "pill" will benefit the general population and reduce unintended pregnancies.

Oral-11

HIGH-THROUGHPUT DRUG SCREENING METHOD FOR DISCOVERING NOVEL CATSPER INHIBITORS

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Presented By: Guillermina Luque, PhD

Introduction & Objective: Sperm hyperactivation requires a rise in intracellular Ca^{2+} brought in by sperm-specific CatSper channels present in the flagellum. The exclusive expression of CatSper in sperm and its critical role in sperm function makes this channel an attractive target for contraception. As a result of the structural and functional complexity of this channel, the strategy of blocking CatSper as a male, non-hormonal contraceptive has not been achieved due to the lack of good screening methods. We aimed to develop a high-throughput method to screen drugs with the capacity to block CatSper: Fluorescent Cell Barcoding.

Methods: For this purpose, we assigned unique fluorescent signatures to different sperm samples as a way to enable higher throughput flow cytometry. These differentially labelled samples incubated in distinct experimental conditions were combined into one tube for simultaneous acquisition, reducing the acquisition time and technical variability, which is essential to perform larger screening experiments for drug discovery using live cells. We employed barcoding flow cytometry in combination with a reporter of membrane potential (Em) $DiSC_3(5)$ in order to determine CatSper function. This assay is based on the fact

that removing external divalent cations by adding EGTA allows CatSper to efficiently conduct monovalent cations, where a sudden influx of Na^+ depolarizes the cell. The magnitude of this depolarization mainly depends on the extent of CatSper opening.

Results: The addition of EGTA resulted in a depolarization of Em in CatSper1 HET sperm due to Na^+ influx through CatSper channels, which did not occur in CatSper1 KO sperm. Secondly, mouse sperm were barcoded with two probes (CellTrace Violet and CellTrace CFSE) that are compatible with the use of propidium iodide (PI) and $DiSC_3(5)$ for viability and Em determination respectively, as their emission spectra do not overlap and can be analyzed easily by flow cytometry. Sperm incubated under different conditions (presence or absence of the CatSper inhibitor HC-056456 10 μM) and stained with different concentrations of CFSE and Violet, were then combined in the same tube for its labelling with PI and $DiSC_3(5)$. We were able to distinguish the sperm samples, and analyze how they respond to the EGTA assay discriminating the live population post-acquisition.

Conclusion: Altogether, these results suggest that we have developed and efficient and simple method to screen drugs with the capacity to block CatSper.

Poster 01

UNDERSTANDING THE ROLE OF UBIQUITIN LIGASE COMPLEX KCTD13-CUL3 IN HUMAN GENITOURINARY DEVELOPMENT

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Presented By: Carolina Jorgez, PhD

Introduction & Objective: Copy number variations (CNV) are an important risk factor for congenital genitourinary defects. One of the most common CNVs involved in congenital genitourinary anomalies is located at the 16p11.2 locus. We identified KCTD13 as an important gene in this region for male lower genitourinary tract defects. KCTD13 acts as a substrate-specific adapter of a BCR (BTB-CUL3-RBX1) E3 ubiquitin–protein ligase complex, which regulates the actin cytoskeleton to modulate cell migration and proliferation. The ubiquitin-proteasome machinery is mainly involved in protein degradation, but also regulates other cellular functions including cell signaling, protein trafficking, cell-cycle progression, and cell death. Reduction of KCTD13 results in elevated RHOA, which positively regulates MAP3K1 and SOX9. These two proteins are important regulators of sexual development and are necessary for the mesenchymal-derived actomyosin contractility required for androgen-driven urethral masculinization. We generated a mouse model lacking *Kctd13* that has cryptorchidism and micropenis with reduced levels of nuclear AR and SOX9 and increased levels of RHOA. We hypothesized that, similar to the mouse model, KCTD13-CUL3 complex in boys may alter the masculinization axes by resulting in inefficient ubiquitination of the masculinization pathways and actomyosin contractility in the urethral mesenchyme leading to abnormal genitourinary development

Methods: Using whole-exome sequencing, we have identified single nucleotide variants (SNV) in KCTD13 that were generated in vitro using site-directed mutagenesis. We generated gain and loss functional studies of KCTD13-specific mutations in genitourinary cell lines. We generated a cell reconstitution ubiquitination assay.

Results: Using whole-exome sequencing, we identified two SNV in KCTD13: E120K and P146L. E120K (binding domain of KCTD13) was identified in a boy with undescended testis (UDT) and hypospadias. The binding of KCTD13 to CUL3 requires the binding of E120 in KCTD13 to R88. The SNV E120K alters the polarity and structure of the BTB domain of KCTD13, causing a change in AR ubiquitination and the formation of actin stress fibers when overexpressed. SNV P146L, which causes a topographic change in the flexible loop of KCTD13, was identified in two boys with UDT. P146L alters the actin stress fiber formation while not affecting the ubiquitination of AR

Conclusion: Our data provides insight into the mechanisms of the KCTD13-CUL3-mediated masculinization pathway, determines the functional consequences of each mutation identified in patients, and elucidates each mutation's association with genitourinary development.

Poster 02

LONG-TERM FOLLOW-UP AFTER HIGH-RISK GONADOTOXIC TREATMENT AND IMMATURE TESTICULAR TISSUE BANKING

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Presented By: Aude Braye, MS

Introduction & Objective: Long-term consequences of oncological treatments become clinically more relevant due to the growing population of long-term childhood cancer survivors. Various malignant and non-malignant conditions are treated by hematopoietic stem cell transplantation (HSCT) or high-risk chemo/radiotherapy (HR-CR) with known long-term effects such as endocrine and gonadal dysfunction. Therefore, fertility preservation programs have emerged to safeguard the future fertility of patients needing high-risk gonadotoxic treatment. For pediatric boys, cryopreservation of immature testicular tissue containing spermatogonial stem cells is currently the only fertility preservation option. Long-term follow-up after high-risk gonadotoxic treatment with or without testicular tissue banking is needed to adapt patients' selection criteria and to better inform patients about their fertility preservation and restoration opportunities. The aim is to evaluate the long-term effects of the high-risk gonadotoxic treatment and the testicular biopsy procedure.

Methods: Pediatric boys treated by HSCT or HR-CR at two Brussels hospitals between 2002 and 2016 and accepting or refusing testicular tissue banking were included. Changes in testicular volume and in the reproductive hormones LH, FSH, testosterone and INHB were evaluated after treatment completion and compared between HSCT

and HR-CR treatment as well as between boys accepting and refusing banking.

Results: Of 60 pediatric boys included, 34 were treated by HCST and 26 required HR-CR. Testicular tissue banking was accepted by 39 boys and refused by 21 boys. Most boys were prepubertal at diagnosis (87%), at treatment completion (78%) and at testicular tissue banking (79%). At the last follow-up visit, 20 boys were still prepubertal (33%) and 40 boys spontaneously initiated or completed their pubertal development (67%). Most patients presented with (sub)normal testicular volumes (86%) and normal LH (81%), FSH (81%), testosterone (70%) and INHB (65%) serum levels at 5.0 (1.0-11.0) years post-treatment. Testicular volumes and LH, FSH and INHB serum levels were not significantly different between the treatment groups. However, significantly more low testosterone serum levels (43%) were recorded after HSCT compared to HR-CR (10%). No significant differences were recorded between the banking groups.

Conclusion: Follow-up data demonstrate more impaired testosterone serum levels after HSCT compared to HR-CR and (sub)normal testicular volumes after high-risk gonadotoxic treatment. The testicular biopsy procedure has no long-term effects on the gonadal development of cancer patients. Longer follow-up studies with a larger study population are needed to confirm these preliminary findings.

Poster 03

ENDOCRINE DISRUPTING CHEMICAL MIXTURES AFFECT THE INTERACTIONS BETWEEN MACROPHAGES AND SPERMATOGONIA

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Presented By: Haoyi Cui, MS

Introduction & Objective: In previous studies, we found that fetal exposure to mixtures of the phytoestrogen genistein (GEN) and the plasticizer di-(2-ethylhexyl) phthalate (DEHP) at doses relevant to human exposure, disrupted the reproduction of male offspring, altering gene and protein expression in several types of testicular cells, including macrophages and germ cells. Thus, our goal was to examine the impact of GEN and MEHP, the main metabolite of DEHP, alone or mixed, on macrophage and spermatogonial cell lines, to understand their potential roles in the effects observed following in vivo exposures.

Methods: Mouse RAW 264.7 macrophage (MΦ) and C18-4 spermatogonia cell lines (spg) were exposed to GEN and/or MEHP at 10^{-5} or 10^{-4} M, either in basal conditions or in the presence of pro-inflammatory lipopolysaccharide (LPS) and cultured separately or together in semi-separated co-culture for 48 h or following 24 h MΦ pre-treatments. Genes/proteins reflecting MΦ and spermatogonial functions were examined.

Results: In co-cultured MΦ, 10^{-4} M GEN and Gen-MEHP mix increased the gene expression of pro-inflammatory *Cxcl2*, *Cxcl10* and *Tnfa* in basal conditions. Interestingly, 10^{-5} M Gen-MEHP mix increased the gene expression of both *Il6* and anti-inflammatory *Il10*, more strongly with LPS. Moreover, GEN alone and Gen-MEHP mix induced the gene expression of *Csf1* in a dose dependent manner in MΦ in co-culture. The addition of MΦ pre-treated with GEN and GEN-MEHP mix to unexposed C18-4 spgs led to decreased expression of *Gfra1*, *Foxo1*, and *Itgb1* in the spgs in a dose dependent manner, in both conditions. Pre-treated MΦ with 10^{-4} M Gen and the mixture also downregulated the gene expression of *Esr1* in spg. This suggests that MΦ pre-treatments altered their behaviors in co-cultures, causing changes in spermatogonia.

Conclusion: The data showing that MΦ pre-exposure to GEN and Gen-MEHP mixtures modified their interactions with spgs, suggest that the effects of EDCs on MΦ could in turn impact spermatogonial functions, possibly contributing to the alterations found in spgs of the testes of rats exposed in utero to GEN and DEHP.

Poster 04

ROHYPNOL INDUCES MALE REPRODUCTIVE TOXICITY VIA SUPPRESSION OF CIRCULATORY ANDROGEN AND UPREGULATION OF CASPASE 3 SIGNALING

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Presented By: David Oluwole

Introduction & Objective: Rohypnol is a benzodiazepine that is used for its anxiolytic, anticonvulsant, muscle relaxant and hypnotic properties, but commonly abused with possible reproductive health consequences. However, little has been documented about its consequences on male reproductive function. Therefore, we evaluated the effect of rohypnol use on testicular function and the possible role of oxidative stress and caspase 3 activity.

Methods: Thirty adult male rats were randomized into six groups of six rats each: the control received 1 mL of distilled water as vehicle, while the low- and high-dose Rohypnol-treated groups received 2 and 4 mg/kg of Rohypnol for 4 weeks. The control-, low- and high-dose recovery groups received 1 mL of distilled water as vehicle and 2 and 4 mg/kg of Rohypnol for 4 weeks respectively, which was followed by a 4-week drug-free recovery period.

Results: Rohypnol treatments led to prolonged mount, intromission and ejaculatory latencies and reduced mount, intromission and ejaculatory frequencies. Also, rohypnol significantly increased post-ejaculatory interval. This was accompanied by reduced circulatory concentrations of FSH, LH, and testosterone, and increased circulatory level of prolactin. Furthermore, rohypnol reduced sperm count, motility, and viability, but increased the percentage of abnormal sperm morphology. These alterations were accompanied by a rise in the markers of testicular injury, oxidative stress, inflammation, and caspase 3-mediated apoptosis. Also, the activities of testicular enzymatic antioxidants and Na^+/K^+-ATPase and Ca^{2+}-ATPase were significantly reduced. Although the impacts of low dose rohypnol treatment were partially reversible, those of the high dose rohypnol treatment were

not. Histopathological findings on the testicular tissues were in tandem with the biochemical alterations.

Conclusion: The findings show that Rohypnol induces reproductive toxicity by suppressing circulatory androgen and Na^+/K^+-ATPase and Ca^{2+}-ATPase activities, and upregulation of caspase 3 signaling through an oxidative-sensitive signaling pathway.

Poster 05

OMEGA 3 FATTY ACID BLUNTS TAMOXIFEN-INDUCED REPRODUCTIVE TOXICITY BY SUPPRESSING OXIDATIVE STRESS, INFLAMMATION AND APOPTOSIS

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Presented By: Moses Hamed

Introduction & Objective: Although the use of tamoxifen (1-[4-(2-dimethyl-aminoethoxy) phenyl]-1,2-diphenyl-1-butene) as an adjuvant therapy in the management of estrogen-dependent breast cancer has been established to be effective, it has been shown to induce organ-specific oxidative damage. Omega 3 fatty acid, on the contrary, has been proven to exert cytoprotection by suppressing oxidative stress. However, there are insufficient studies documenting the effects of tamoxifen and omega 3 fatty acid, when used singly or in combination, on male reproductive function. Thus, we evaluated the effect of tamoxifen and/or omega 3 fatty acid on male sexual behavior, steroidogenesis and spermatogenesis. The roles of oxidative stress, inflammation, and apoptosis were also probed.

Methods: Thirty-two inbred litter-mate adult male Wistar rats were randomized into four groups (n = 8/group), vehicle-treated, tamoxifen-treated, omega 3 fatty acid-treated, and tamoxifen + omega 3 fatty acid-treated. Administration was once daily via oral route and lasted for 4 weeks.

Results: Tamoxifen significantly impaired penile reflexes and male sexual competence. In addition, there was a remarkable rise in the testicular activities of gamma glutamyl transferase and lactate dehydrogenase, and lactate, glucose, and cholesterol concentrations, but a decline in sorbitol dehydrogenase activity in tamoxifen-treated rats. Also, tamoxifen caused a reduction in the serum levels of GnRH, FSH, LH, testosterone, and dopamine and testicular testosterone, 3β-HSD and 17β-HSD as well as sperm quality. The noted tamoxifen-induced biochemical derangements aligned with the testicular histopathological findings (distorted seminiferous tubules, reduced leydig cell mass, reduced luminal spermatozoa, and reduced spermatogenic index). Furthermore, tamoxifen reduced testicular antioxidants (glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase). This was accompanied by a rise in markers of testicular oxidative stress (uric acid, xanthine oxidase, malondialdehyde, 8-OHdG), inflammation (myeloperoxidase activity, TNF-α, IL-6), and apoptosis (caspase 3). These observed tamoxifen-led alterations were abolished by omega 3 fatty acid administration.

Conclusion: This study showed that omega 3 fatty acid confers protection against tamoxifen-induced reproductive dysfunction by suppressing the oxidative stress, inflammation and apoptosis.

Poster 06

ROLE OF BASAL CELL PRIMARY CILIA FROM THE EPIDIDYMIS IN THE CONTROL OF TISSUE REGENERATION AFTER INJURY

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Presented By: Laura Girardet

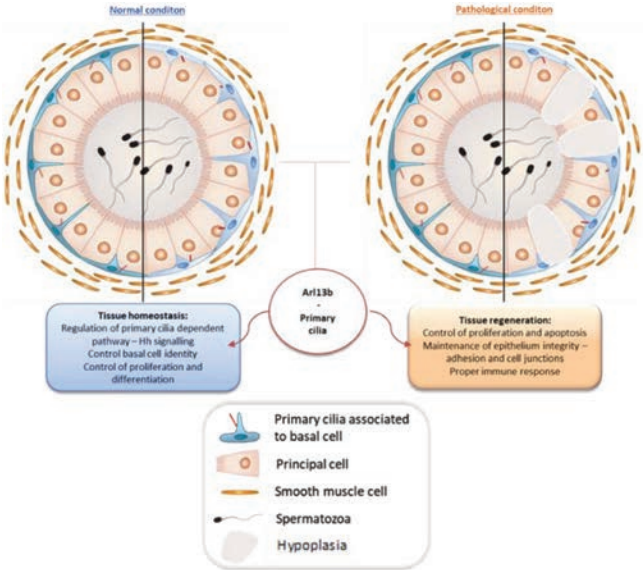
Introduction & Objective: The primary cilium (PC) is a signaling antennae that controls cellular functions through the transduction of Hedgehog (Hh) signaling and whose dysfunction causes multisystemic diseases called ciliopathies. In the adult epididymis, PCs are exclusively found associated with basal cells, which displays stem cell like properties. Acknowledging that PC control cellular self-renewal and differentiation in other tissues, we hypothesized that PC could participate to epididymis homeostasis, through the regulation of basal cell Hh signaling.

Methods: To study PC functions in vivo, we impaired PC-dependent Hh signaling through the deletion of the ciliary GTPase Arl13b in keratin5-positive basal cells (cKO model). We validated that both PC length and Hh signaling pathway were impaired in cKO compared to control (Ctl) mice according to immunofluorescent detection of PC, quantitative PCR and western blot on Hh target genes.

Results: Although male cKO mice were fertile, the expression level of epithelial cells markers was substantially altered, with a reduction by 60% ($p < 0.05$) of KRT5, KRT14, and P63 basal cell markers and a two-fold increase of AQP9 and CFTR principal cell markers. While this witnessed an imbalanced cellular organization in cKO mice, possibly due to impaired basal cell differentiation, we assessed the capacity of the epithelium from cKO mice to regenerate following injury. To this aim, efferent ductules ligation (EDL) was performed on cKO and Ctl mice to induce a transitory wave of apoptosis. While the epithelium regenerates after EDL in Ctl mice, increased apoptosis and decreased proliferation rates were observed in the proximal epididymis of cKO vs. Ctl mice (by two-fold and a three-fold, respectively, $p < 0.001$), according to Caspase3 and PCNA staining. This imbalanced response was associated with hypoplasia in the epithelium of cKO mice, evidencing the impaired capacity of the epithelium to regenerate. The expression of more than 646 genes was significantly modified following EDL in cKO vs. Ctl mice (ANOVA,$Fc > 1.2$;$p < 0.05$), including genes that are important players in immune response, differentiation or adhesion.

Conclusion: Altogether, our results indicate that Arl13b participates to the transduction of the Hh signaling pathway in basal cells and is necessary for regeneration of the epididymis following injury. These data

suggest that the impaired ciliogenesis, observed in ciliopathic patients, may not only affect epididymis homeostasis but also its capacity to regenerate after a trauma.



Basal cell Arl13b functions in the epididymis

Poster 07

SEGMENTAL DIFFERENTIATION OF THE MURINE EPIDIDYMIS: IDENTIFICATION OF SEGMENT-SPECIFIC, GM1-ENRICHED EXTRACELLULAR VESICLES AND REGULATION BY LUMINAL FLUID FACTORS

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Presented By: Danielle Sosnicki, BS, MS

Introduction & Objective: Sperm maturation in the epididymis is essential for natural fertilization, yet surprisingly, we know very little about the physiology or function of this organ. The organization of the epididymis varies across species, with the mouse having distinct segments, well-demarcated by connective tissue septae. These segments provide opportunity to identify specific epididymal functions and the mechanisms by which this organ effects changes in sperm. Improved understanding of epididymal maturation could enhance treatment of male infertility, improve assisted reproductive technologies, and conversely, provide new targets for male contraceptives. Here, we investigated segmental expression of a bioactive lipid, the ganglioside G_{M1}. This glycosphingolipid is known to play key roles in sperm capacitation and acrosome exocytosis and is used as a biomarker for sperm fertilizing ability in human clinical medicine.

Methods: Direct fluorescence labeling of G_{M1} was performed on epididymal tissue sections from wildtype (WT) mice and imaged via confocal microscopy to localize G_{M1}. To determine if G_{M1} originated from the epididymis or from the testis, and to determine whether that expression depended upon signals from developing male germ cells, azoospermic mouse models (Msh4$^{-/-}$ and Trip13$^{Gt/Gt}$) were examined. Efferent duct ligation (EDL) procedures were also performed on WT mice and epididymides were collected 3 weeks post-EDL and examined.

Results: G_{M1} was highly expressed in segment 2 of the murine caput epididymis, with relatively reduced expression in the rest of the organ. Confocal microscopy revealed that G_{M1} was present in principal cells and clear cells, as well as luminal extracellular vesicles (EVs). In both azoospermic models, segmental differences were lessened including expression of G_{M1}, which was particularly increased in segment 1. Epididymides of mice that had EDL procedures also had loss of segment-specific G_{M1} expression, looking similar to the azoospermic mouse models.

Conclusion: Our results indicate that the G_{M1}-enriched EVs originated from the epididymal epithelium (not testis or sperm). We hypothesize that these EVs are a population of epididymosomes involved in sperm maturation. These combined data suggest that segmental differentiation of G_{M1} expression depends upon exposure to an unknown factor in the luminal fluid that originates from the testis and depends on spermatogenesis. Together, our findings provide an entry point for functional, mechanistic studies to improve understanding of epididymal function and male fertility.

Poster 08

CX3CR1 DEFICIENCY LEADS TO IMPAIRMENT OF THE IMMUNE SURVEILLANCE IN THE EPIDIDYMIS

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Presented By: Ferran Barrachina, BS, MS, PhD

Introduction & Objective: Mononuclear phagocytes (MPs) play an active role in the immunological homeostasis of the epididymis. We previously showed that intraepithelial and interstitial CX3CR1^{+}MPs can internalize and process circulatory antigens. Here, we examined whether the lack of functional CX3CR1 in homozygous mice (CX3CR1$^{EGFP+/+}$) alters the ability of MPs to initiate immune responses.

Methods: Confocal microscopy and flow cytometry were used to characterize the immunophenotype of epididymal MPs in CX3CR1$^{EGFP+/-}$ (control) and CX3CR1$^{EGFP+/+}$ mice. Antigen capture was assessed 1 h after i.v. injection of ovalbumin-Alexa 647 (OVA). Immune response was also evaluated 48 h after intravasal-epididymal injection of saline and lipopolysaccharide (LPS) from *E. coli*.

Results: Intraepithelial MPs located in the epididymal initial segments (IS) from CX3CR1$^{EGFP+/+}$ mice displayed a significant reduction in the number of luminal-reaching projections ($p < 0.01$) compared to control, and the remaining projections were in close interaction with clear cells, similarly to control. Confocal microscopy showed

that most CX3CR1$^{EGFP+/+}$ and control MPs were co-labeled with the macrophage marker (F4/80). Flow cytometry showed no differences in the percentage of macrophages (F4/80$^+$) or dendritic cells (DC; MHCII$^+$CD64$^-$) between CX3CR1$^{EGFP+/+}$ and control MPs. Flow cytometry showed no overall impairment in OVA internalization in CX3CR1$^{EGFP+/+}$MPs, but confocal microscopy revealed reduced OVA capture specifically in intraepithelial CX3CR1$^{EGFP+/+}$ cells from the IS compared to control. In addition, while CX3CR1$^{EGFP+/+}$MPs showed a reduction in the percentage of cells with a monocytic phenotype (Ly6C$^+$MHCII$^-$CD64$^-$) ($p < 0.05$) versus control under physiological conditions, a higher number of MPs with a monocytic signature was observed following LPS injection. Interestingly, we found CD103$^+$ cell accumulation in the CX3CR1$^{EGFP+/+}$ cauda epididymis after LPS injection ($p < 0.05$), suggesting that deletion of CX3CR1 affects antigen transfer and results in the inability of CD103$^+$DCs to migrate. We also revealed cell-cell interactions between MPs and CD103$^+$DCs in CX3CR1$^{EGFP+/+}$ and control epididymis.

Conclusion: Our study reveals morphological alterations in luminal intraepithelial projections in MPs from CX3CR1-deficient mice, which displayed an impaired antigen sampling, and an immunophenotypic shift in response to injury. Additionally, our results indicate that CX3CR1 deletion induces defective cell-cell communication between MPs and CD103$^+$DCs and support the hypothesis that MPs are the gatekeepers of the immunological blood-epididymis barrier by surveying antigens. By identifying immune mechanisms required for antigen sampling and presentation, our study may lead to new therapies for male infertility associated with immunological disorders.

Poster 09

GENERATION OF NR5A1-INDUCIBLE HUMAN-LEYDIG LIKE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Presented By: Lu Li

Introduction & Objective: Primary hypogonadism is based on the lack or reduced response of Leydig cells to luteinizing hormone (LH) affecting their ability to synthesize testosterone (T) in humans. Leydig cell transplantation is a reasonable solution for treating primary hypogonadism. We previously reported that human Leydig-like cells (hLLCs) could be induced from human induced pluripotent stem cells (hiPSCs) using transient transfection of NR5A1 expression vectors in combination with DHH, db-cAMP, and hCG. However, T formation by hLLCs can only be maintained in vitro for limited time. Our goal is to develop a hLLC model that maintains the long-term ability to produce T.

Methods: We established NR5A1-inducible hLLCs from hiPSCs that allow for the induction of expression of NR5A1 in response to doxycycline (Dox). The generation of the NR5A1 product steroidogenic factor 1 (SF-1) was monitored by fluorescence. hiPSCs carrying the NR5A1-inducible system (iN-hiPSCs) were induced into NR5A1-inducible hLLCs (iN-hLLCs) following our previously reported methods (Li et al., PNAS 2019; 116:23274-23283). Basal and hormone-treated iN-hLLC T was measured by ELISA. FACS was used to enrich for cells expressing high levels of SF-1.

Results: The NR5A1-inducible system was successfully inserted into the genome of hiPSCs. Compared to previously reported hLLCs, iN-hLLCs have a robust response to hCG stimulation in terms of T production that can be maintained in vitro for at least 8 weeks. When increasing doses of Dox (0, 1000, 2000, 4000 ng/mL) were added to the medium, T formation by iN-hLLCs increased in a dose-dependent manner. After FACS sorting, we obtained three groups of cells with different intensities of fluorescent signals: high expression of SF-1 (high-intensity) group, low expression (low-intensity) group, and negative (no-signal) group. The high-intensity group can synthesize T at much higher levels than the low-intensity group and for longer periods of time (12 weeks) in culture.

Conclusion: The iN-hLLCs induced from iN-hiPSCs present an extended and stable capacity to synthesize T compared to hLLCs. This makes them a promising source for use to restore T levels in hypogonadal animal models in vivo. Moreover, the NR5A1-inducible system enables us to manipulate the T biosynthetic capacity of iN-hLLCs and establish high and low T producing Leydig cell models for further assessment of the molecular pathways mediating these phenotypes.

Poster 10

A KNOCK-IN MOUSE MODEL FOR MALE FERTILITY: BASIS FOR THE MAMMAL-SPECIFIC PROTEIN PHOSPHATASE ISOFORM PP1Y2 IN SPERM

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Presented By: Wesam Nofal, BS, MS

Introduction & Objective: Mammalian testicular sperm are immotile, gaining motility during their passage through the epididymis. Motility hyperactivation takes place in the female reproductive tract. These processes, essential for male fertility, are unique to mammals. Protein kinases and phosphatases are responsible for phosphorylation of proteins involved in normal sperm function. Knock out of these signaling enzymes in mice result in impaired male fertility. The phosphatase PP1γ1 and PP1γ2, are alternately spliced transcripts derived from one gene, *Ppp1cc*. They are identical, except at their extreme C-termini. Splicing and formation of PP1γ2 occurs only in mammals. Sperm from non-mammalian species and monotremes contain one of the PP1 isoforms α,β ,or γ 1. *Ppp1cc* knockout male mice are infertile due to impaired sperm morphogenesis and function. Transgenic expression of PP1γ2, but not PP1γ1, driven by an exogenous testis specific promoter, rescues sperm formation and fertility of mice lacking *Ppp1cc*. The purpose of the study is to development of a mouse model for the determination of biochemical mechanisms of how PP1γ2 functions in sperm which will enable understanding of male fertility and possible causes for infertility.

Methods: Because spermatozoa lacking PP1γ2 cannot be made we have modified the *Ppp1cc* gene by Crisper/Cas9 such that only the PP1γ1 isoform will be expressed. The knock in mice were validated by gene sequencing and PCR. Western blot and immunocytochemistry were used to determine PP1γ1 protein in sperm and testis. In vivo breading was used to determine fertility of these mice. High-speed video recording and flagellar beat analysis were used to ascertain how motility is impaired in the KI mice.

Results: We have successfully established validated breeding lines to generate homozygous (KI/KI) mice. Localization of PP1γ1 showed that its localization within sperm was dramatically altered compared to PP1γ2 in WT sperm. Motility analysis showed that forward motility was reduced and flagellar beat amplitude was dramatically suppressed. Fertility of the KI mice was severely impaired.

Conclusion: The PP1γ1-bearing mouse should be a valuable model to determine why mammals require PP1γ2, while spermatozoa from non-mammalian species function normally with the other PP1 isoforms. Differences in the phosphoproteins and metabolic functions of spermatozoa from wild type compared to the PP1γ1 KI mice valuable in understanding how PP1γ2 functions to maintain normal spermatozoa function and male infertility.

Poster 11

EFFECT OF INTERLEUKIN-1β ON THE DEVELOPMENT OF DIFFERENT STAGES OF SPERMATOGENESIS FROM SPERMATOGONIAL CELLS OF NORMAL AND BUSULFAN-TREATED IMMATURE MICE IN VITRO

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Presented By: Mahmoud Huleihel, PhD

Introduction & Objective: Interlukin-1β (IL-1β) is a cytokine that produced by all testicular cells. It is known to induce proliferation of germ cells and to affect Sertoli and Leydig cell functions. Busulfan is a chemotherapy that affects proliferating cells in the testes, and thus may lead to infertility. We demonstrated a significant decrease in the expression levels of IL-1b in the testis of busulfan-treated immature mice (unpublished data). One of the options for fertility preservation of prepubertal boys, before chemotherapy, is the development of spermatogenesis from their testicular stem cells in vitro.

Objectives: To evaluate the effect of IL-1β on the capacity of spermatogonial cells, from busulfan-treated and normal immature mice, to develop spermatogenesis in vitro.

Methods: Busulfan-treated immature mice (7 days old ICR) were intraperitoneally (i.p) injected with busulfan (45 mg/kg). Mice were sacrificed after 10 days for in vitro culture. Cells from seminiferous tubules (STs) of busulfan-treated or normal immature mice, were enzymatically isolated. These cells were cultured in methylcellulose (MCS) [three-dimension (3D) culture system] in the absence or presence of IL-1β (1,10,100 pg/mL). Fresh media without (control) or with IL-1β were added from the beginning of the culture and after 2 weeks. Cells were collected after 5 weeks, and examined for pre-meiotic (VASA), meiotic (BOULE) and post-meiotic (ACROSIN) cells by immunofluorescence staining or qPCR.

Results: Addition of IL-1β to MCS with cells from STs of normal immature mice significantly increased the percentages and expression levels of VASA, BOULE and ACROSIN-positive cells, in a dose-dependent manner. Furthermore, IL-1b significantly increased the expression levels of androgen receptor and transferrin, without effect on androgen binding protein and FSH-R compared to control. However, even though addition of IL-1b to MCS that contained cells from STs of busulfan-treated immature mice significantly increased the expression levels of VASA, BOULE and ACROSIN compared to their own control, those levels were lower compared to cultures from normal mice.

Conclusion: Our findings indicate the involvement of IL-1β on the proliferation and differentiation of spermatogonial stem cells in vitro. IL-1b may directly and/or indirectly affect spermatogonial cell development. The low effect of IL-1β on the development of spermatogenesis, from busulfan-treated mice in MCS, compared to normal mice may suggest impairment in the functionality of spermatogonial and supporting cells following chemotherapy, and thus lead to infertility.

Poster 12

SPERM DNA IDENTIFICATION USING WHOLE MOLECULE ANALYSIS OF TARGETED BISULFITE SEQUENCING DATA

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Presented By: Ryan Barney, MS

Introduction & Objective: Each cell type is known to vary in amount and distribution of DNA methylation. The ability to utilize this unique feature to identify the cell of origin of a molecule of DNA could have important clinical and forensic applications when specifically targeting sperm such as pre-screening patients prior to a micro-TESE procedure. Identify unique sperm DNA methylation patterns that can be distinguished from somatic cell patterns using next generation sequencing.

Methods: DNA Methylation array data from 12 individuals were used to identify over 40,000 regions of differential methylation between pure sperm DNA and seminal cell-free DNA. Four DNA regions know to have a unique sperm signature were then selected and analyzed in six individuals (somatic cells and sperm) using a targeted sequencing approach with Illumina's iSeq instrument. The regions analyzed contained at least CpGs which were all expected to be methylated in sperm DNA and not methylated in blood DNA. Methylation status was evaluated at each of the identified CpGs using a custom python-based pipeline.

Results: The initial comparison of methylation status resulted in a distinct difference between DNA from sperm and somatic cells. We found all amplicons in each individual to be highly discriminatory between sperm and somatic cells. At one of the targeted sites which

only contains two CpGs, 100% of DNA molecules with both CpGs methylated were found in sperm samples and 99.7% of DNA molecules with both CpGs unmethylated were found in the somatic tissue. This was assessed in over 10,000 reads per region for both sperm and somatic cells. Other sites followed similar trends.

Conclusion: The initial results show a very distinct difference between spermatozoa and somatic DNA methylation patterns. Further analysis of more samples at several regions is expected to refine a well-defined DNA methylation pattern unique to spermatozoa. This sperm DNA methylation signature will provide the ability to produce an efficient identification assay of sperm DNA that could have broad clinical and forensic applicability.

Poster 13

TRANSGENIC MOUSE MODELS OF HUMAN GENE VARIANTS ASSOCIATED WITH NON-OBSTRUCTIVE AZOOSPERMIA

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Presented By: Jimmaline Hardy, MS, PhD

Introduction & Objective: Approximately 1% of men and 10% of infertile men suffer from azoospermia or absence of sperm in the ejaculate. Non-obstructive azoospermia (NOA), without physical impairment to the reproductive tract, accounts for ~60% of azoospermia diagnoses. Genetic errors are estimated to cause > 50% of all NOA cases. Our group identified a large deletion in *TEX11*, a testis-specific meiotic gene, associated with human NOA. Subsequent analyses of unrelated patients identified multiple point mutations, presumed to result in NOA. To date, *TEX11* variants are posited to account for ~2% of NOA cases yet validation of pathogenicity and functional mechanism have yet to be determined. Given the limited availability of testicular tissue from affected individuals, we have utilized a transgenic mouse model approach to demonstrate causality of single gene point mutations and indels in NOA.

Methods: Genetic variants were identified from the blood of azoospermic individuals using array comparative genomic hybridization and whole exome sequencing. CRISPR/Cas9 technology was used to introduce three likely pathogenic variants, one missense, one splicing, and one frameshift leading to a premature stop, into the homologous gene of DBA/2 mice. Fertility status, testis morphology, seminiferous tubule histology, and chromosome structure were assessed using standard and advanced molecular techniques.

Results: At 12 weeks of age, each of the three variants produced testicular phenotypes that partially mimicked the human condition. The frameshift variant resulted in infertility due to complete meiotic arrest, which most strongly resembled that in humans though at a different stage of prophase I. Both point mutations did not result in infertility at this age though chromosomal abnormalities were seen. Thus, subfertility may be the corresponding manifestation.

Conclusion: The human and mouse genomes are ~85% homologous overall. Our investigation highlights the applicability of transgenic mouse models for validating experimentally identified genetic variants associated with NOA. The disparity in spermatozoa production observed with human variants versus the mouse complement could be attributed to differences in reproductive biology, for example, the influence of species specific TEX11 on accompanying genes' expression. Alternatively, mutant *TEX11* induced NOA may be a progressive disorder with increasing number of spermatogenic cycles having a more severe impact. Long-term analysis of germ cell dynamics in mouse may provide additional insight into the consequence and mechanism of identified variants.

Poster 14

UNDERSTANDING THE EFFECTS OF SPERM METABOLISM LEADING TO IMPROVED FERTILIZATION AND EMBRYO DEVELOPMENT

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Presented By: Ana Romarowski, PhD

Introduction & Objective: Sperm capacitation involves a fine crosstalk between metabolic and signaling pathways. Recently, the Visconti group developed a technique where all nutrients were removed from the medium, sperm were incubated until become motionless, and nutrients were added back. This method, named Sperm Energy restriction plus Recovery (SER) significantly improved the percentage of hyperactive sperm, in vitro fertilization and embryo development rates. The aim of this work was to understand the effect of SER treatment on sperm at the metabolomic and molecular level.

Methods:

- CASA measurements.
- IVF.
- Glucose consumption measurements.
- Seahorse technology measurements.
- NMR and Mass Spectrometry.
- ATP measurements.
- Western Blot.

Results: We found that recovering with only glucose as energy substrate was sufficient to produce the increased hyperactivation and IVF levels obtained after SER treatment, while recovering with only pyruvate was not. In addition, SER with only glucose treated sperm showed that glycolysis and oxidative phosphorylation levels were recovered. Following ^{13}C-labeled glucose metabolites, 2D ^{1}H-^{13}C HSQC NMR experiments showed that citrate is significantly higher in capacitated

sperm than in non- capacitated sperm. Moreover, we found that sperm intracellular ATP levels were recovered after SER treatment. Our NMR data show that there is a distinct metabolite profile observed in 1D ^{1}H NMR spectra for each treatment and the most significant differences are seen in the starving condition.

Conclusion: Glucose alone was sufficient to produce the increased hyperactivation and IVF levels obtained after SER treatment, while pyruvate alone was not, suggesting that the glycolytic pathway is necessary to be active for SER improvement on sperm function. Moreover, because all these experiments were conducted only in the presence of glucose (^{13}C-citrate is derived from ^{13}C-glucose), they suggest that pyruvate (or lactate) produced during glycolysis in the sperm principal piece is able to migrate to the mitochondria mid-piece and enter the Krebs cycle. In addition, SER treated sperm showed recovered glycolysis and oxidative phosphorylation levels. Consistently, we found that ATP levels were similar to non SER sperm. Interestingly the most significant differences were seen in the starving condition. These results show that the metabolites changed at the starving step could explain why these sperm have improved functionality. Altogether, these results indicate that manipulation of sperm metabolism can affect sperm function improving the outcome of embryo development.

Poster 15

HIGH ANTI-SPERM ANTIBODY LEVELS ARE NOT ASSOCIATED WITH PREGNANCY OUTCOMES AFTER VASECTOMY REVERSAL

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Presented By: Catherine Nam, MD

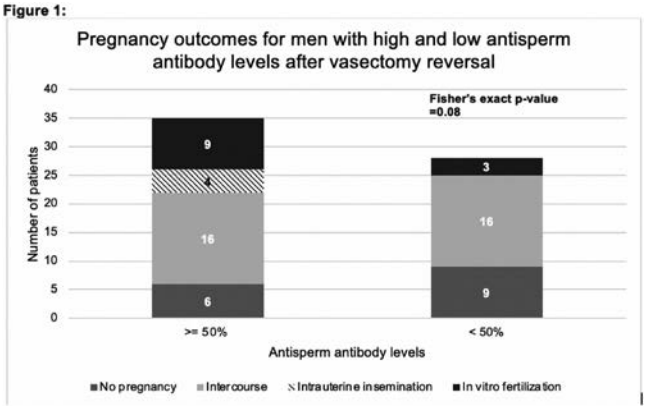
Introduction & Objective: After vasectomy reversal, many men wonder if antisperm antibodies (ASAs) will prevent them from getting pregnant and American Urological Association vasectomy guidelines call for additional research in this area. Therefore, we examined the relationship between ASA levels following vasectomy reversal and pregnancy outcomes.

Methods: Our lab performs IgG ASA testing on all semen samples with progressive motile sperm concentrations of ≥ 2 M/mL. Therefore, we did a retrospective chart review and phone interview of patients who underwent vasectomy reversal at our institution from January 2000 to December 2018. We excluded patients who underwent vasectomy reversal for pain and patients with progressive motile sperm concentration < 2 M/mL. From the chart review, we extracted patient demographics, surgical and semen analyses data. On the phone interview, we asked for pregnancy outcomes and method of conception. We used the last sperm count within one year post operatively to categorize men as having low (< 50%) or high ASA (≥ 50%) levels, based on the reference range in our lab. Our primary outcome was pregnancy rate, including method of conception. Differences in pregnancy rates were tested using a Fisher's exact test.

Results: 195 men met our inclusion criteria. Median age at time of surgery was 40.1 years and median obstruction interval was 7.3 years. Median partner age was 31.8 years. One hundred fifty-six (80%) of patients underwent bilateral vasovasostomy, 35 (18%) underwent vasovasostomy and vasoepididymostomy, one (0.5%) underwent bilateral vasoepididymostomy, and three (1.5%) underwent unilateral vasovasostomy. Median ASA levels were 68% [Interquartile range (IQR) 10-95%]. 63 men also completed phone interviews. Demographics were similar between men who did and did not complete phone interviews (all p-values ≥ 0.07). There were 35 men with high ASAs and median ASA levels were 93% (IQR 85-97%). There were 28 men with low ASAs and median ASA levels were 7% (IQR 0-14%). Figure 1 displays the pregnancy outcomes of the men with high and low ASA levels. The Fisher's exact test p-value was 0.08 for differences in pregnancy outcomes.

Conclusion: ASA levels are not associated with pregnancy outcomes or method of conception after vasectomy reversal. These findings can improve patient counseling before and after vasectomy reversal.

Figure 1:



Poster 16

A SOLUBLE ADENYLYL CYCLASE INHIBITOR PROVIDES ON-DEMAND, NON-HORMONAL CONTRACEPTION IN MALE MICE

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Presented By: Melanie Balbach, PhD

Introduction & Objective: There are no non-hormonal, pharmacological methods for contraception, an important public health gap further exacerbated by a dearth of contraceptive options for men. Soluble adenylyl cyclase (sAC: ADCY10) is essential for male fertility and specifically and reversibly blockable by small molecular inhibitors so it is an ideal target for a male contraceptive.

Methods: Using structure-assisted drug design we developed and screened for sAC inhibitors with nanomolar potency and good systemic

availability and characterized them in vitro in mouse and human spermatozoa and ex vivo and in vivo in male mice.

Results: The sAC inhibitors block capacitation, acrosome reaction, and motility of cauda epididymis-isolated mouse sperm with high potency in vitro and also interrupt these processes in ejaculated human spermatozoa, where sAC activity has already been stimulated by the bicarbonate concentrations found in semen. A single injection of these inhibitors into male mice after one hour even after high dilution inhibits ex vivo activation of sperm with bicarbonate, reversibly blocks sperm motility and prevents female pregnancies in a restricted mating study.

Conclusion: This provides the proof-of-principle that pharmacological sAC inhibition provides non-hormonal, on-demand male contraception.

Poster 17

THE ROLE OF CLOMIPHENE CITRATE IN OPTIMIZING SPERM COUNT, CONCENTRATION, AND MOTILITY IN INFERTILE MEN

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Presented By: Tristan Chun, BS

Introduction & Objective: Clomiphene Citrate (CC) is an estrogen modulator supported by the AUA for off-label use in the treatment of male hypogonadism and oligospermia. CC acts on the hypothalamic-pituitary-gonadal axis, ultimately stimulating testicular testosterone production. CC modulates spermatogenesis by stimulating FSH secretion and optimizing the testosterone:estrogen ratio, and therefore may provide fertility benefits. This investigation aimed to evaluate the efficacy of CC in improving semen parameters of oligospermic men.

Methods: 532 men were identified from our institutional database from 2010 to 2020 undergoing at least two semen analyses; 80 subjects met inclusion criteria. This study included infertile men above the age of 18 with oligospermia who received with 25 mg CC daily or every other day and underwent a semen analysis before and after treatment (per WHO guidelines). Semen parameters, demographics, and co-morbidities were recorded. Statistical analysis was conducted through R computational language and all tests were two-sided using a significance level of 0.05.

Results: The median age of our cohort was 34 years (IQR 32-40). Patient comorbidities included 11% hypertension, 9% diabetes mellitus, and 9% hyperlipidemia. Of note, 20% of men in this cohort had erectile dysfunction and 54% had concurrent hypogonadism. CC treatment demonstrated a statistically significant increase of $p < 0.0001$ in sperm count [3.85(0-15.28) to 16(3.4-48)] X10ˆ6, sperm concentration [1.4(0.01-5.23) to 5.2(1.3-19.4)] X10ˆ6/mL, and percent of motile sperm [29.5%(10-41) to 38%(29-53)]. Sperm morphology was not significantly changed with CC treatment. The percentage normal midpiece morphology increased from 74% to 75% ($p < 0.05$) and percentage with head abnormality increased from 45% to 47% ($p < 0.05$). Other semen parameters such as semen volume, white blood cell concentration, or immature germ cell concentration were comparable before and after CC treatment. Subset analysis yielded QD was not superior to QOD CC frequency.

Conclusion: In men with primary infertility and oligospermia, CC has the propensity to significantly improve semen parameters, specifically sperm count, concentration, and motility; yet sperm morphology was largely unchanged. In summary, CC optimizes sperm quantity and improves sperm motility, but has limited effect on morphology. Our data supports the use of Clomiphene Citrate as a practical therapy for improving semen parameters in the infertile man with oligospermia.

Variable	Prior to Tx	After Tx	Diff	Paired P value
Volume mL (IQR)	2.9 (1.55 – 3.5)	2.7 (2 – 4)	-0.2	0.29
Sperm Count million (IQR) x10^6/mL	3.85 (0 – 15.28)	16 (3.4 – 48)	+12.1	<0.0001
Sperm Concentration (IQR)	1.4 (0.01 – 5.23)	5.2 (1.3 – 19.4)	+6.6	<0.0001
Percent Motile (IQR)	29.5 (10 – 41)	38 (29 – 53)	+8.5	<0.0001
WBC Concentration (%)	0 (0 – 1.3)	0.6 (0 – 1.9)	+0.6	0.12
Immature Germ Cell Concentration (%)	0 (0 – 1.3)	0.75 (0 – 1.9)	+0.75	0.07
Percent Sperm Morphology (IQR)	—	—	—	—
– Normal Sperm	17.5 (10 – 22)	18 (10 – 24)	+0.5	0.44
– Head Abnormality	45 (0 – 52.2)	47 (26.5 – 53)	+2.0	0.03
– Midpiece Abnormality	21 (0 – 25.25)	22 (14.7 – 25.3)	+1.0	0.70
– Tail Abnormality	5 (4 – 6)	6 (5 – 6)	+1.0	0.29

Table 1: Semen Parameter Modulation with Clomiphene Citrate

Poster 18

COMBINED ANTIBIOTIC THERAPY IN MEN WITH LEUKOCYTOSPERMIA AND MODULATORY EFFECT ON SEMEN ANALYSIS

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Presented By: Tristan Chun, BS

Introduction & Objective: Empirical treatment options available for the management of infertile men with leukocytospermia have been a topic of controversy. This is largely because it is not uncommon for patients without fertility issues to have leukocytes present on semen analysis. However, prior studies reported patients with infertility and isolated leukocytospermia subjected to antibiotic treatment have displayed improved pregnancy rates. In this study we evaluated the efficacy of the triple antibiotic regimen of vibramycin 100 mg BID/2 weeks + ciprofloxacin 100 mg BID/2 weeks + gentamicin 80 mg injection x1, in modulating semen parameters.

Objective: To investigate the efficacy of a triple antibiotic therapy in infertile men with leukocytospermia.

Methods: A database of 532 patients receiving at least 2 semen analyses from 2010 to 2020 at our single institution was reviewed for this retrospective study. Inclusion criteria were as follows: drug naive men presenting for initial treatment with infertility over the age of 18,

WBC > 1x10ˆ6/mL on semen analysis as per WHO 2010 guidelines, combined treatment of vibramycin 100 BID, ciprofloxacin 100 BID, and gentamicin 80 mg injection x1, and full data with pre/post antibiotic semen analyses. Semen parameters were compared through R computational language and all tests were two-sided using a significance level of 0.05.

Results: A total of 103 men met the inclusion criteria with an average age of 33 (IQR 29 – 37). Patients in our cohort had minimal comorbidities with eight (8%) hypertensive, two (2%) dyslipidemic, and one patient with type 2 diabetes. After triple antibiotic therapy WBC concentration significantly decreased from a median of 2.6 (1.9 – 4.45) to 1.9 (1.3 – 3.2) X 10ˆ6/mL with a paired p value < 0.0001. Additionally, patients displayed a significant increase ($p < 0.05$) in sperm parameters such as total motility [38% (26.3 – 44.8) to 40% (28 – 51.3)] and percent normal sperm morphology [15% (12 – 21) to 18% (12 – 22)]. All other semen parameters including volume and sperm concentration were not statistically different from baseline after treatment.

Conclusion: These results support the combined use of vibramycin, ciprofloxacin, and gentamicin in the treatment of infertile men with leukocytospermia. The triple antibiotic combination was effective at decreasing seminal WBC concentration and improving sperm motility and progression. We are currently conducting further analysis into these significant improvements, in hopes of assessing changes in pregnancy rates.

Variable	Prior to Tx	After Tx	Diff	Paired P value
Volume mL (IQR)	2.6 (1.95 – 3.6)	2.7 (2 – 3.95)	+0.1	0.41
Sperm Count million (IQR)	43.5 (16 – 106)	49 (15.7 – 91.3)	+5.5	0.89
Sperm Concentration (IQR)	16.2 (5.96 – 36.8)	14 (5.6 – 33.2)	-2.2	0.42
Percent Motile (IQR)	38 (26.3 – 44.8)	40 (28 – 51.3)	+2	0.007*
WBC Concentration 10^6/mL (IQR)	2.6 (1.9 – 4.45)	1.9 (1.3 – 3.2)	-0.7	<0.0001***
Immature Germ Cell Concentration (%)	2.6 (1.8 – 3.9)	2.6 (1.7 – 3.9)	0	0.73
Percent Sperm Morphology (IQR)	---	---	---	---
– *Normal Sperm*	15 (12 – 21)	18 (12 – 22)	+3.0	0.0007**
– *Head Abnormality*	50 (43.5 – 54)	49 (44 – 53)	-1.0	0.21
– *Midpiece Abnormality*	26 (24.5 – 28.5)	27 (25 – 29)	+1.0	0.19
– *Tail Abnormality*	6 (5 – 7)	6 (5 – 6)	0	0.92

Table 1: Semen Parameters. *p < 0.0001

Poster 19

CLOMIPHENE CITRATE TREATMENT RESPONSE OF SEMEN PARAMETERS AND TESTOSTERONE LEVELS IN HYPOGONADAL MEN OVER TIME

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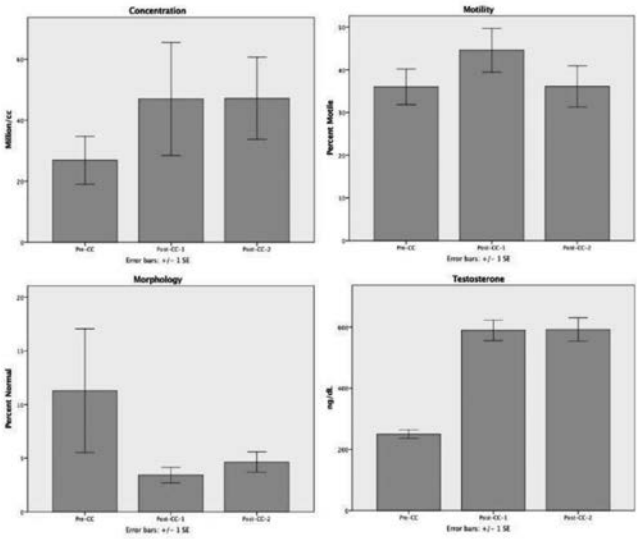
Presented By: Michael Basin, MD

Introduction & Objective: Clomiphene Citrate (CC) has been shown to improve total testosterone (TT) and sperm parameters in hypogonadal men. However, duration of treatment with CC until peak effect is unknown. Since timing until optimal results after CC treatment is unclear, we sought to evaluate TT levels and semen parameters in hypogonadal men treated with CC over time.

Methods: We performed a retrospective review of consecutive hypogonadal men with idiopathic infertility treated with CC between 2015 and 2021. Men were included if pre-treatment and at least two post-treatment semen analyses (SA) and TT levels were available. Patients with coexisting endocrine disorders, azoospermia, secondary infertility, prior testicular disorders, and T supplementation were excluded. Statistical analysis was performed using repeated measures ANCOVA with Bonferroni correction for pairwise analysis and Huynh-Feldt correction for sphericity.

Results: Twenty-one patients were included. Median age was 33 (25-44) years and mean body mass index (BMI) was 34.8 (SD 8.4). Mean pre-treatment TT was 250.1 (SD 64.5). Mean time between initiation of CC and first post-treatment analysis was 3.7 months (SD 1.1), while mean time between first and second post-treatment analysis was 6.1 months (SD 5.3). On pairwise analysis, TT levels significantly increased following initiation of CC to post-CC-1 (250.1 vs. 589.3, $p < 0.0001$) and post-CC-2 (250.1 vs. 593.2, $p < 0.0001$). Yet, TT levels remained stable between post-CC-1 and post-CC-2 ($p = 1.0$). Increase in semen concentration did not reach significance on pairwise analysis. However, on repeated measures ANCOVA, controlling for age, BMI, smoking status, letrozole use, and varicocoeles, semen concentration was significantly improved ($p = 0.032$) and there was no significant change in serum testosterone ($p = 0.312$).

Conclusion: For this cohort, optimal response to CC in hypogonadal men with idiopathic infertility occurred at three months. Statistically significant TT improvement was noted after 3 months, with significant sperm concentration improvement taking more time, likely due to the smaller sample size and larger standard deviation. Larger studies are warranted to evaluate if a 3-month time interval yields the optimal TT and sperm concentration response rates for most men treated with CC.



Effect of Clomiphene on Semen Parameters and Testosterone

Poster 20

USE OF MELATONIN AND CAFFEINE IN CRYOPRESERVATION PROCESS BY SLOW-FREEZING METHOD INCREASES POST-THAW SPERM MOTILITY

Juliana Pariz[1,2], Larissa Chiba[2], Mayara Rodrigues[1,2], Heloisa Faquineti[2,3], Ana Clara Barduchi[2], Mabel Schulz[4], Joel Drevet[5], Raul Sanchez[4], Jorge Hallak[2,3]

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Presented By: Juliana Pariz, PhD

Introduction & Objective: Cryopreservation processes can damage spermatozoa and impair structural and functional cell characteristics, and these injuries depend on the initial semen characteristics and the method selected for cryopreservation. Knowing the behavior of semen samples cryopreserved by different techniques, using substances that guarantee viable sperm for post-thaw fertilization is a challenge for Andrology and Cryobiology. The objective of this study is to compare the effects of melatonin and caffeine supplementation on the quality of post-thaw spermatozoa cryopreserved by slow freezing and vitrification methods, using normozoospermic and asthenozoospermic semen samples.

Methods: This prospective study included 54 semen samples from male voluntaries between January 2020 and November 2021. Basic semen analysis was performed in the fresh sample and forwarded for cryopreservation by two methodologies: slow-freezing and vitrification. Antioxidant substance (2 mM melatonin) was added in the pre-cryopreservation solution, and stimulant substance (2 mM caffeine) was added in post-thaw samples. All samples were submitted before cryopreservation and after thawing to seminal analysis and complementary tests, such as DNA fragmentation, mitochondrial activity, and reactive oxygen species (ROS). Sperm cryosurvival rate was calculated after slow-thawing and devitrification. Samples were grouped according to initial characteristics: progressive motility (PR) $\geq$32% (normozoospermia; NOR) and PR < 32% (asthenozoospermia; AST). The Student's t-test was used to compare means. Grant: Fapesp (2019/18571-0).

Results: Were included 37 normozoospermic samples [mean of age = 32.19 years-old; Standard Deviation (SD) = $\pm$7.28] and 17 asthenozoospermic samples (mean of age = 33.07$\pm$11,29). In the NOR group cryopreserved by slow-freezing method, the melatonin and caffeine supplementation increased post-thawed total ($p = 0.037$) and progressive ($p = 0.003$) motility; devitrification with supplementation did not show significant improvement. In the AST group cryopreserved by slow-freezing method, the supplementations increased post-thawed progressive motility ($p = 0.004$), total motility ($p = 0.002$), and cryosurvival rate ($p = 0.011$); and reduced ROS levels ($p = 0.017$). Slow-freezing was less harmful in the seminal parameters evaluated than vitrification in NOR and AST groups.

Conclusion: Caffeine and melatonin in sperm samples has proven to be a very effective and simple way to improve semen motility, both in normozoospermic and asthenozoospermic samples. This will be particularly useful for initial low-quality semen samples cryopreserved by the slow-freezing method, those which suffer the most from the freezing/thawing process.

Poster 21

ASSESSMENT OF GENOTOXICITY OF VAS-OCCLUSIVE MALE CONTRACEPTIVE ON SPERM

Kevin Eisenfrats, Rucha Bhat

Contraline, Inc., Charlottesville, VA, USA

Presented By: Kevin Eisenfrats

Introduction & Objective: Vas-occlusive contraceptives are devices that are implanted into the vas deferens for long-term occlusion of sperm with the potential to be more easily reversible than vasectomy. Chromosomal mutations in sperm are key indicators of safety and reversibility for a novel male contraceptive. Contraline's vas-occlusive contraceptive, known as ADAM, is an injectable hydrogel that has shown to be biocompatible and biodegradable. To our knowledge, this is the first study which utilized fluorescence in-situ hybridization (FISH) to assess the genotoxicity of a vas-occlusive hydrogel and its degradation products on sperm.

Methods: Hydrogel extracts were prepared by incubating hydrogel implants for 72 hours at 37°C in phosphate-buffered saline (PBS). Degradation products were prepared by incubating hydrogel implants for 30 days at 70°C in PBS (previously shown to degrade the implant). Semen samples from 6-12 healthy, fertile donors were pooled and purified. Purified sperm were then incubated for 24 hours at 37°C with either hydrogel extracts, degradation products, or left untreated. Human spermatozoa survival assay (HSSA) testing was performed and showed no decrease in sperm motility when incubated with the hydrogel extract or degradation products compared to negative control (media only). Chromosomes X, Y, 13, 18, and 21 (key chromosomal markers for fertility) were marked with fluorescent probes, and an automated imaging system was used to categorize normal vs. abnormal sperm.

Results: A generalized linear regression framework was used to analyze the percentage of mutation present in each sample. Overall, there was a low observed rate of any chromosomal aberration (< 5% across all samples). There was no significant change in mutation rates of 13 Disomy, 18 Disomy, 21 Disomy, Diploid Sperm, or Sex Chromosome Disomy from the hydrogel extract ($\alpha = 0.05$) or the degradation products ($\alpha = 0.05$).

Conclusion: It was concluded that the vas-occlusive hydrogel implant and its degradation products would not increase the risk of sperm genetic abnormalities. This data corroborates previously conducted genotoxicity studies, which demonstrated no structural chromosomal aberrations to human lymphocyte cells when exposed to the ADAM hydrogel and degradation products.

Poster 22

PRE-PUBERTAL CODEINE EXPOSURE EXAGGERATES MATERNAL CODEINE EXPOSURE-INDUCED DELAY IN PUBERTY ONSET AND SEXUAL DYSFUNCTION IN MALE WISTAR RAT: POSSIBLE ROLE OF ARGININE

Roland Akhigbe, Oladele Afolabi, Ayodeji Ajayi

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Presented By: Roland Akhigbe, MBBS

Introduction & Objective: Codeine, a common drug of abuse, has been reported to be a potential risk factor for male infertility. On the other hand, L-arginine has been shown to enhance male reproductive function. However, little is known about the long-term consequences of maternal and pre-pubertal exposure to codeine and L-arginine on male reproductive health. Therefore, we assessed the impact of maternal and pre-pubertal exposure to codeine in combination with L-arginine on puberty attainment and sexual competence in subsequent generation of male rats and evaluated their effects on androgen receptor gene expression (mRNA AR).

Methods: Forty pre-pubertal female Wistar rats were randomized into two cohorts (n = 20/group), vehicle-treated and codeine-treated, and administered 0.5 mL of distilled water and 5 mg/kg of codeine via gavage respectively from post-natal day (PND) 28. The animals were then paired with stud male Wistar rats of comparable age and weight (1: 1) at 12 weeks old. Administration continued throughout pregnancy and lactation period. Male Offsprings were weaned at PND 21 and each cohort was randomized into vehicle treated (0.5 mL distilled water), codeine-treated (5 mg/kg of codeine), L-arginine-treated (300 mg/kg of arginine), and codeine with L-arginine-treated (5 mg/kg of codeine + 300 mg/kg of arginine). Intervention commenced at PND 28 and lasted for 8 weeks.

Results: Codeine significantly reduced the anogenital distance (AGD) and anogenital index (AGI) at PND10 and PND22. Also, animals damned by codeine-exposed mothers showed delayed preputial membrane separation which was further prolonged by pre-pubertal codeine-exposure. In addition, penile reflexes and mount, intromission and ejaculation frequencies were significantly reduced by codeine exposures, while mount, intromission and ejaculation latencies were increased by codeine exposures. Furthermore, codeine exposures reduced the motivation to mate and increased post-ejaculation interval. These alterations were accompanied by reduced circulatory levels of FSH, LH, testosterone, dopamine, and NO and penile cGMP. Also, codeine exposures reduced testicular AR mRNA expression. However, the observed codeine-induced alterations were blunted by L-arginine treatments.

Conclusion: Thus, we demonstrated that maternal exposure to codeine exerts a negative transgenerational effect on male puberty attainment and sexual function via suppression of testosterone and dopamine as well as downregulation of NO/cGMP signaling. This was worsened by a combined pre-pubertal codeine exposure in animals damned by codeine-exposed mothers. Nonetheless, L-arginine treatment attenuated codeine-induced sexual dysfunction.

Poster 23

SLEEP DISORDERS ARE ASSOCIATED WITH HYPOGONADISM AND ERECTILE DYSFUNCTION - A U.S. CLAIMS DATABASE ANALYSIS

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Presented By: Pranjal Agrawal

Introduction & Objective: Along with diet and exercise, sleep is considered the third pillar of health. Numerous studies have observed that insufficient sleep and/or disrupted sleep due to sleep disorders can result in poor overall health, including adversely affecting men's health and sexual function. However, most of these studies have been limited by sample size and/or performed at single institutions. Our objective was to assess the association of various sleep disorders with erectile dysfunction (ED) and hypogonadism using a large national claims database.

Methods: A US health research network (the TriNetX Diamond Network) of over 190 million patients, encompassing prescriptions and healthcare encounters, was queried from 2009 to 2021. Amongst men aged 40-70 years, insomnia (ICD-10 G47.0), sleep apnea (G47.33), and circadian rhythm sleep disorder (G47.2) were each independently assessed to determine the association with testicular hypofunction and ED. A matched control cohort for age, obesity, hypertension, hyperlipidemia, diabetes mellitus, and ischemic heart disease was generated excluding those with any sleep disorders (G47 & F51), sleep deprivation (Z72.820), or morbid obesity with alveolar hypoventilation (E66.2). Propensity-score matching analysis was performed between the two groups

Results: 2,086,389 men with insomnia, 3,748,323 men with sleep apnea, and 83,560 men with circadian rhythm sleep disorder were identified with an equivalent number of propensity-score matched control men. (Table 1) Men with insomnia had higher rates of testicular hypofunction (OR 1.89 [1.88- 1.91]) and ED (OR 1.46 [1.45-1.47]) compared to matched controls. Those with sleep apnea were also more likely to have testicular hypofunction (OR 1.70 [1.68-1.71]) but had only slightly higher rates of ED (OR 1.09 [1.08- 1.09]) compared to matched controls. Finally, men with circadian rhythm dysfunction had a very high association with testicular hypofunction (OR 3.1 [3.0-3.2]) and ED (OR 1.6 [1.5-1.7]) compared to matched-controls.

Conclusion: In this large analysis based on US claims, we showed sleep disorders, especially circadian rhythm dysfunction, to be strongly associated with hypogonadism and ED. It is thus crucial for urologists to screen for poor sleep when conducting a thorough work-up for

hypogonadism and ED, to catch these underlying diseases in order to provide patients with the optimal treatment for complete health.

	Insomnia		Sleep Apnea		Circadian Rhythm Dysfunction	
	Sleep Disorder	*Matched Cohort*	*Sleep Disorder*	*Matched Cohort*	*Sleep Disorder*	*Matched Cohort*
Included Men	2,086,389	2,086,389	3,748,323	3,748,323	83,560	83,560
% Testicular Hypofunction (E29.1)	9.3%	5.1%	8.3%	5.1%	12.5%	4.4%
Odds Ratio (95% Confidence Interval)	1.89 (1.88-1.91)		1.70 (1.68-1.71)		3.08 (2.96-3.20)	
% Erectile Dysfunction (N52)	12.5%	8.9%	9.4%	8.7%	12.3%	8.1%
Odds Ratio (95% Confidence Interval)	1.46 (1.45-1.47)		1.09 (1.08-1.09)		1.60 (1.55 - 1.66)	
Average Age	57.1 ± 8.6	57.1 ± 8.6	57.4 ± 8.34	57.4 ± 8.34	54.6 ± 8.6	54.6 ± 8.6
% Obesity (E66)	19.4%	19.4%	20.6%	20.6%	29.2%	29.2%
%Hypertension (I10-16)	50.6%	50.6%	44.1%	44.1%	44.8%	44.8%
%Hyperlipidemia (E78)	45.7%	45.7%	38.2%	38.6%	43.1%	43.1%
%Diabetes Mellitus (E08-13)	19.8%	19.8%	19.4%	19.2%	17.2%	17.2%
%Ischemic Heart Disease (I20-25)	11.6%	11.6%	10.3%	10.3%	8.8%	8.8%

Association among Sleep Disorders and Men's Health Diagnosis

Poster 24

VENO-OCCLUSIVE ERECTILE DYSFUNCTION'S COMBINED TREATMENT WITH SURGERY, AUTOLOGOUS STEM CELLS AND VEGF

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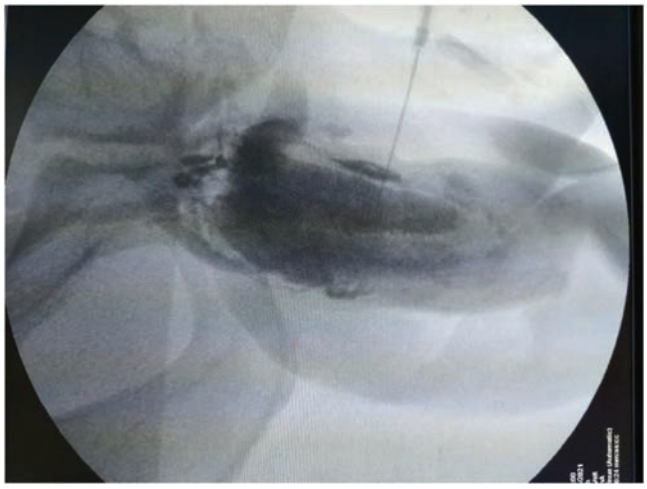
Presented By: Oleksandr Knigavko, MD, PhD

Introduction & Objective: Insufficiency of venous obstruction of the corpora cavernosa under the tunica albuginea is the main cause of veno-occlusive erectile disfunction (VOED) in young and middle-aged men. The aim of the study is to determine the effectiveness of complex treatment of VOED using injections of autologous mesenchymal stem cells (ASC) and VEGF in combination with veno-occlusive surgery.

Methods: 2012-2021 in Andrology department KRMCCUN 197 patients with VOED were examined and treated. One hundred thirty-nine (70.5%) patients were diagnosed with left-sided or bilateral varicocoele. The average age of patients was 36.5 ± 4.1 years. Patients with prostatitis or depression/anxiety were excluded from the study or previously treated. The patients were divided into three groups: group I consisted of 57 (28.9%) patients with distal form of VOED, group II-78 (39,6%) with proximal form of VOED, group III - 62 (31.5%) patients with combined form. Patients from group I were embolized deep dorsal and plexus veins of Santorini through deep dorsal vein. For group II - Marmara operation from peno-pubical approach was combined with ligation of deep dorsal and veins of the penis. For patients of group III both methods were performed. Veno-occlusive surgery patients made up subgroup A-156 (79.2%) people. Forty-one (20.9%) patients additionally received ASC therapy 2 million five times (two times before and three times after surgery) every 1.5-2 months, VEGF 15 times every 10 days by intracavernous injections composed subgroup B. During occlusive surgery, 2 million ASC were injected into ligated veins in a retrograde direction. We compared results twice in 2 and 10 months after treatment.

Results: Due to the subjective data of the IIEF-5 questionnaire the veno-occlusive surgical treatment effectiveness after 2 months was practically the same in both groups: 16.7 ± 3.1 and 16.9 ± 3.0. After 10 months, the results were significantly higher in group B than in A: 19.6 ±. 3.4 and 14.3 ± 3.2. ACS and VEGF injections significantly improve erectile function under the doppler ultrasound scan of the penis (absence of venous leak), in 34 (82.9%) in group B compared with the results of group A 88 (56.4%).

Conclusion: Adding ACS and VEGF microinjections to venoocclusive surgery significantly improves effectiveness of treatment VO erectile disfunction



Combine form of VOED on cavernosography

Poster 25

SUPERFICIAL PENILE VEIN THROMBOPHLEBITIS AFTER MICROSURGICAL VARICOCELECTOMY

Moshe Wald, Fiona Armstrong-pavlik
University of Iowa, Iowa City, IA, USA
Presented By: Moshe Wald, MD

Introduction & Objective: Varicocoele is an abnormal dilation and tortuosity of the internal spermatic veins within the pampiniform plexus of the spermatic cord. Varicocoele is a common condition, estimated to be present in 15% of the general male population. Surgical intervention for varicocoele repair may be considered in cases of scrotal pain and infertility associated with impaired sperm parameters. Microsurgical varicocelectomy is currently a common method for varicocoele repair. This study assessed the incidence of superficial penile vein thrombophlebitis after microsurgical varicocelectomy, as well as possible risk factors for its development.

Methods: A retrospective chart review was conducted. Charts of patients who underwent microsurgical varicocelectomy by a single surgeon at an academic medical center between May 2005 and June 2020 were reviewed. Excluding those lost to follow up, the cohort had 408 patients. Each chart was examined to determine if a patient had experienced thrombophlebitis or thrombosis of superficial penile veins after surgery, and whether patients had the following hypothesized risk factors: clotting disorders, hypertension, diabetes, or a past history of thrombophlebitis elsewhere.

Results: The average age of patients in the study was 33.5 years (± 9.3 years). Of the 408 patients screened, 5 (1.2%) had thrombophlebitis (2) or thrombosis (3), with an average age of 29.3 years (± 5.7 years). Given the small number of patients who developed these conditions, no further statistical analysis was performed. Notably, though, none of these patients had hypothesized risk factors such as clotting disorders, hypertension, diabetes, or a history of thrombophlebitis elsewhere.

Patients were treated with twice-daily naproxen (one patient, 8 weeks), daily aspirin (3 patients: 3 weeks (1) or 6 weeks (2)), or without medications (one patient). The average treatment time was 6.6 (± 0.9) weeks, after which patients' symptoms had resolved (four patients) or they were lost to follow-up (one patient).

Conclusion: Clotting in superficial penile veins following microsurgical varicocelectomy is a rare occurrence. Further study is needed to better understand the risk factors for thrombophlebitis in this region as well as the best way to treat it.

Poster 26

ESTABLISHING THE STANDARD VALUES OF SHEAR-WAVE ELASTOGRAPHY OF PEDIATRIC TESTIS

Abinav Udaiyar[1], Robert Wilson[2], Stanley Kogan[1,3], David Childs[4], Marc Colaco[3], Marshall Schwartz[1,3], Steve Hodges[1,3], Anthony Atala[1,3], Hooman Sadri-Ardekani[1,3]

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Presented By: Abinav Udaiyar, BS

Introduction & Objective: Shear-wave elastography (SWE) is a technique that measures sonographic quantification of tissue elasticity. Previous studies provide evidence of apparent structural and histological differences in testicular tissue elasticity for certain testicular diseases. SWE provides a noninvasive investigation of the stiffness of the testicular tissue as a primary diagnostic tool for certain testicular disorders with the potential to replace biopsies and exploratory surgical procedures. While studies are investigating normal shear wave elastography values for testis in adult men, no studies have provided standardized normal range values for shear wave elastography for the pediatric testis. The purpose of this study is to establish the normal threshold ranges of shear-wave velocity in pediatric patients (0 – 18 years).

Methods: Patients from one clinic location were screened for the study, excluding those with a known history of testicular diseases, sex chromosome abnormalities, or testicular surgery. Sixty-one volunteers (mean age: 6.29 ± 4.55, range: 2 months – 18 years) were chosen and underwent routine B-mode sonography and simultaneous multi-frame shear-wave elastography of both testes. Patients were divided into prepubertal and peripubertal age groups: 3 months – 9 years and 10 – 18 years (n = 45 and n = 16 respectfully). The volume of each testis and the shear-wave velocity at the testis' superior, center, and inferior poles were measured and collected.

Results: The mean shear-wave velocity for the patients under and over 10 years old was 1.24 ± 0.21 and 1.0989 ± 0.26 m/s respectively (p = 0.0076, ± SD, Figure 1D). However, when age groups were analyzed by anatomical pole, a statistically significant decrease in the shear-wave velocity was found between groups at the inferior, central, and superior pole with p -values of 0.0389 (1A), 0.0002 (1B), and 0.0228 (1C), respectively.

Conclusion: From the 61 patients, preliminary data to determine normal shear-wave velocities for a pediatric testis population was collected. Significant decreases in shear-wave velocities found between the age groups at each anatomical pole may indicate a pubertal effect on tissue elasticity as the testes mature and grow. These results demonstrate the necessity to establish a separate pediatric normal standard for SWE of the testis. We will continue to enroll more patients to better define standards for comparison.

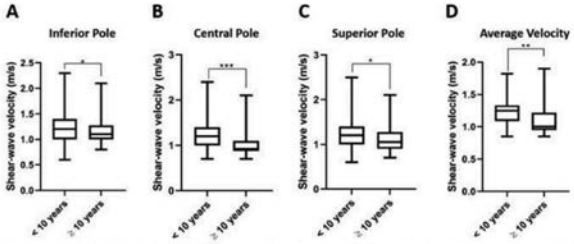

Figure 1. Shear-wave velocities were measured at the (A) inferior, (B) central, and (C) superior poles of the testes, and used to calculate (D) the average shear-wave of the whole testis. Mann-Whitney U test significance (* = p < 0.05; ** = p < 0.01; *** = p < 0.001).

Shear-wave elastography results

Poster 27

CYTOMEGALOVIRUS IN SEMEN

Alexander Hauser, Ann Kiessling, Mark Hartley, Maureen Kearnan

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Presented By: Alexander Hauser, BS

Introduction & Objective: Current sperm donor guidelines do not include direct testing of semen specimens for cytomegalovirus (CMV), the leading cause of hearing and vision loss in newborns in the U.S. Antibody status (IgM or IgG) is not a reliable predictor of active CMV shedding into semen. The objective of the current study is to measure the frequency of CMV DNA in the semen of men donating sperm for IVF.

Methods: All semen specimens from men donating sperm for family planning are RT-PCR tested for HIV and CMV. 0.5 mL of each specimen is added to sufficient guanidium hydrochloride crystals with polyA carrier and EDTA stabilizer to equal approximately 3.5 M guanidium hydrochloride, a concentration shown to lyse virus and somatic cells, but not sperm heads, which are pelleted by centrifugation. Nucleic acids are purified from the guanidium supernatant through Qiagen columns and analyzed by RT-PCR for HIV RNA and proviral DNA, and CMV DNA. HIV-infected men provide test results for the most recent CD4+ lymphocyte count, and paired blood specimens are tested for CMV IgG and IgM.

Results: Four hundred twenty-nine donors submitted 1238 semen specimens, of which 1029 were tested for CMV by PCR amplification and HIV by Reverse Transcription PCR; paired serum was tested for CMV IgG/IgM. Three hundred fifty-five samples tested positive for CMV, and 291 tested positive for HIV. One hundred forty-seven donors submitted at least one CMV-positive specimen, while 178 submitted all CMV-negative specimens.

Donors submitting at least one positive specimen had an average CD4+ count of 730, while donors presenting all negative specimens had an average CD4+ count of 724. There was no correlation between specimens testing positive for HIV and CMV.

Conclusion: CMV is present in the semen of HIV-infected men more frequently than HIV. The presence of CMV in semen is underappreciated. Serology will not accurately predict the presence of CMV in semen, a known transmitter of the virus.

Poster 28

CAPACITATION-INDUCED REGULATION OF GSK3 ALPHA SUGGESTS THAT THIS KINASE IS INVOLVED IN THE ACROSOME REACTION

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Presented By: Gayatri Mohanty, PhD

Introduction & Objective: The multifaceted protein glycogen synthase kinase (GSK3) that exists in alpha and beta isoforms is known to play an essential role in sperm capacitation and male infertility. cAMP pathway is one of the major pathway that regulates sperm capacitation. With the immediate exposure of the sperm to the capacitation media, influx of bicarbonate (HCO3-) ions and the efflux of cholesterol through bovine serum albumin (BSA) as acceptor induces the soluble adenyl cyclase and its downstream effectors. In the current study we therefore investigated the regulation of GSK3 alpha and beta during capacitation.

Methods: Caudal epididymal mouse sperm collected from CD1 males were incubated in conditions that support or not capacitation. GSK3 alpha and beta isoforms and the phosphorylated versions of these kinases were visualized using Western blotting and immunofluorescence. In addition, loss of function experiments were conducted using lithium and CHIR99021 to inhibit GSK3 activity. Amongst the functional assays, we measured motility patterns using CASA and the acrosome reaction.

Results: We observed that capacitation was associated with activation of GSK3 alpha and redistribution of this kinase in the head region. On the other hand, we did not observe changes in the phosphorylation status and localization of GSK3 beta. Interestingly, GSK3 alpha phosphorylation have a biphasic kinetics, while increased phosphorylation was observed at the starting of the process, it was then dephosphorylated. GSK3 inhibitors block the acrosome reaction without affecting intracellular calcium.

Conclusion: These results indicate differential regulation and localization of GSK3 alpha and beta. Because phosphorylation of Ser21 (for GSK3 alpha) and Ser9 (for GSK3 beta) is associated with inactivation of these kinases, our results suggest that GSK3 alpha increased its activity as part of the capacitation process and that this activation is restricted to the sperm head. The GSK3 alpha activation appears to be compartmentalized in the sperm head. Dephosphorylation of GSK3 alpha and the effect of GSK3 inhibitors blocking the acrosome reaction suggest that this kinase plays a role in the regulation of this exocytotic event.

Poster 29

MALE FERTILE POTENTIAL ASSESSMENT AMONG HIGH AND LOW INTENSITY CYCLISTS

Luana Adami, Valter Maciel Junior, Ricardo Bertolla
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Presented By: Luana Adami

Introduction & Objective: Introduction: Physical exercise practice has been recommended as a means to improve quality of life (QOL). Specifically, cycling promotes cardiovascular protection; when intensely practiced, however, it may lead to injuries or undesired side-effects, such as decreasing the male fertile potential due to testicular impact and heat and mechanical shock, harming both the gametes and the seminal microenvironment, as well as promoting erectile dysfunction. The practice of cycling has been associated with changes in semen quality. However, the comprehension of sperm function is still unclear. Cycling can have recreational or sport purposes, but also can be routinely used as alternative transport. Thus, considering the investment of large cities in non-polluting transport policies and alternative urban mobility, such as bicycles, there is a need to very this impact on semen and sperm quality in men. Objective: This study aimed to verify the effects of intense cycling on male fertile potential.

Methods: The prospective study included 72 semen samples from High-Intensity group (ride more than 8 hours/week, HI, n = 32), Low-Intensity group (less than 8 hours, LI, n = 28) and Control group (no cyclists, n = 12). Volunteers were evaluated by a urologist and then referred for seminal collection. A semen aliquot was used for conventional evaluation (WHO 2010), another was centrifuged, resuspended in Biggers Whitten Whittingan culture medium, and analyzed for kinetic analysis, acrosome integrity, mitochondrial activity, mitochondrial membrane potential assessment, DNA fragmentation (DFI), and sperm intracellular superoxide anion activity (SOD). Statistical analysis was performed using SPSS 21.0, starting with descriptive data analysis and followed by Generalized Linear Models tests. *Gamma* and *Normal* data distributions and the *Akaike Information Criteria* (AIC) were adopted for model adequacy. Statistical significance was set at 0.05.

Results: The LI group had significantly higher rates of neutrophils, velocity average path (VAP), velocity on a straight line (VSL), and sperm DNA integrity when compared to the HI group. On the other hand, the HI group had significantly higher rates of SOD and DFI when compared to the LI group, as well as higher DFI when compared to Control group

Conclusion: These results allow us to conclude that high-intensity cycling is associated with an increase in sperm DNA fragmentation, concomitant with an increase in intracellular oxidative activity.

Table. Sperm evaluation parameters including motility, intracellular superoxide anion activity, DNA fragmentation, between High Intensity, Low Intensity and Control groups.

Variable	High Intensity (n=32)	Low Intensity (n=28)	Control (n=12)	*p*
Neutrophils(10^6/mL)				
Mean; SD	0,20; 0,28[a]	1,16; 1,48[b]	0,48; 0,55[a]	0,0001
CI (95%)	0,11 – 0,35	0,72 – 1,87	0,25 – 0,94	
SOD (%)				
Mean; SD	35,15; 17,62[a]	32,09; 14,12[b]	22,43; 3,46	0,008
CI (95%)	29,52 – 41,85	27,25 – 37,79	16,56 – 30,36	
VAP (µm/s)				
Mean; SD	40,84; 0,39[a]	40,86; 0.37[b]	40,97; 0.44	0,003
CI (95%)	40,69 – 40,99	40,72 – 41,00	40,71 – 41,23	
VSL (µm/s)				
Mean; SD	34,38; 10,11[a]	37,21; 8,78[b]	28,36; 9,79	0,007
CI (95%)	30,87 – 37,90	33,95 – 40,47	22,81 – 33,91	
DFI I (%)				
Mean; SD	7,51; 10,17[a]	8,59; 8,94[b]	19,33; 9,54	0,0001
CI (95%)	3,97 – 11,06	5,27 – 11,90	13,92 – 24,74	
DFI III (%)				
Mean; SD	22,08; 12,59[a]	21,48; 10,73[b]	13,00; 6,95	0,003
CI (95%)	18,11 – 26,93	17,84 – 25,86	9,60 – 17,60	
DFI IV (%)				
Mean; SD	1,94; 2,54[a]	3,53; 2,22	1,33; 2,38[b]	0,007
CI (95%)	1,06 – 2,83	2,70 – 4,36	0,02 – 2,68	

SOD- superoxide anion activity; VAP – velocity average path; VSL – velocity on a straight line; DFI - DNA fragmentation; Data are presented in: Mean, SD (standard deviation) e CI (Confidente Interval); different superscript letters on the same line indicates a post hoc *test significant difference*

Sperm evaluation parameters including motility, SOD and DFI.

Poster 30

EFFECTS OF DIFFERENT CONCENTRATIONS OF MELATONIN ON SPERM FUNCTIONAL TESTS AND SEMINAL OXIDATIVE PROFILE POST CRYOPRESERVATION: INITIAL REPORT

Larissa Chiba[1,2], Juliana Pariz[3], Heloisa Faquineti[1], Raul Sanchez[4], Joel Drevet[5], Mabel Andrea Rubilar Schulz[4], Jorge Hallak[1,2,6,7]

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Presented By: Larissa Chiba, BS

Introduction & Objective: Cryopreservation has been widely used to preserve fertility, although it can have damage to sperm function and characteristics, mainly the high generation of reactive oxygen species (ROS), leading to reduced mitochondrial activity and increased DNA fragmentation. In order to reduce these injuries, studies using melatonin are being carried out, however, the efficacy of this substance and its role in the functional control of spermatozoa are not well established in the literature. This study aimed to determine melatonin concentration to improve sperm quality, analyzing the sperm functional tests and ROS levels after cryopreservation in the slow freezing method.

Methods: Fifty-five seminal samples from voluntary men (18-45 years old) with seminal volume ≥1.5 mL and sperm concentration ≥15 million/mL were used in this study. After initial analysis, samples were cryopreserved by slow-freezing method with TEST-YOLK buffer (TYB), as a cryoprotectant, added to 0.01 , 2 , 3 mM of melatonin, and without supplementation, as the negative control (CONT). Seminal parameters, DNA integrity (SCSA®), reactive oxygen species (luminol chemoluminescence), mitochondrial activity (DAB), and lipid peroxidation (TBARS assay) were evaluated before and after cryopreservation. Cyosurvival rates were calculated post-thawing. Comparisons among groups were analyzed with a one-way analysis of variance (ANOVA).

Results: The results are demonstrated in Table 1. The p-value was not statistically significant between groups except for the cryosurvival rates ($p < 0.05$). Looking at the averages, lipid peroxidation and DNA fragmentation seem to have improved in samples with 2 mM, whereas samples supplemented with 0.01 mM improved in ROS, mitochondrial activity, and cryosurvival rates compared to the negative control.

Conclusion: This study demonstrated an initial benefic effect of melatonin supplementation as a protectant agent in samples cryopreserved by slow freezing, preserving or improving semen quality post-thaw, however in this cohort we did not find any significant differences between the groups therefore it is necessary to continue the study to confirm the benefits of the melatonin.

	Negative control	0.01mM	2mM	3mM	p-value
Total motile sperm number (million/mL)					
Mean; SD	**2.83; 6.73**	1.61; 2.62	1.60; 3.37	1.53; 2.44	0.303
Min-Max	0,00-48.00	0.00-13.46	0.00-17.00	0.00-13.50	
Progressive motility (%)					
Mean; SD	**6.71; 7.60**	5.41; 7.84	5.74; 7.41	6.25; 7.76	0.825
Min-Max	0.00-27.49	0.00-38.26	0.00-39.24	0.00-28.40	
Total motility (%)					
Mean; SD	**14.63; 10.98**	11.53; 10.64	11.14; 10.69	12.07; 12.53	0.378
Min-Max	0.00-42.42	0.00-48.31	0.00-53.17	0.00-69.00	
ROS (10^4cpm/million spermatozoa)					
Mean; SD	1.78; 4.49	**1.51; 3.83**	3.07; 8.37	3.39; 11.37	0.549
Min-Max	0.00-24.17	0.00-18.59	0.00-41.16	0.00-74.02	
DNA fragmentation (DFI%)					
Mean; SD	61.44; 21.35	62.88; 25.86	**57.98; 23.57**	58.19; 24.82	0.672
Min-Max	2.00-100.00	11.00-100.00	17.00-100.00	20.00-100.00	
Mitochondrial activity (DAB I+II%)					
Mean; SD	59.50; 18.56	**65.00; 19.05**	62.00; 7.00	54.00; 15.23	0.824
Min-Max	44.00-81.00	43.00; 76.00	54.00-67.00	34.00-70.00	
Cryosurvival (%)					
Mean; SD	32.42; 39.92	**36.20; 48.38**	19.45; 18.47	22.79; 23.60	0.047
Min-Max	0.00-266.00	0.00-252.50	0.00-90.12	0.00-100.97	
Lipid Peroxidation (TBARS/ml nanograms of spermatozoa)					
Mean; SD	415.27; 175.50	651.66; 551.56	**27.28; 24.92**	196.79; 154.68	0.184
Min-Max	291.17-539.37	261.64-1041.67	0.00-48.84	87.41-306.16	

Mean; Standard Deviation (SD); Min-Max: Minimum and maximum values; significant p-value <0.05.

Table 1. Results post-thawing with and without melatonin.

Poster 31

EJACULATED HUMAN SPERMATOZOA CYCLE BETWEEN STATES OF FERTILITY AND INFERTILITY, THE KNOWLEDGE OF WHICH CAN PREDICT IUI SUCCESS

Clifford Librach[1], Sergey Moskovtsev[1], Peter Wieckowski[2], Junyan Zhang[1], Kimberly Bingham[2], Amy Coffey[2], John Walsh[3], Zimu Chen[3], Paul Turek[4], Erin Schnellinger[5], Ronald Parkinson[3], Barb Cohen[3], Joseph Hill[2]

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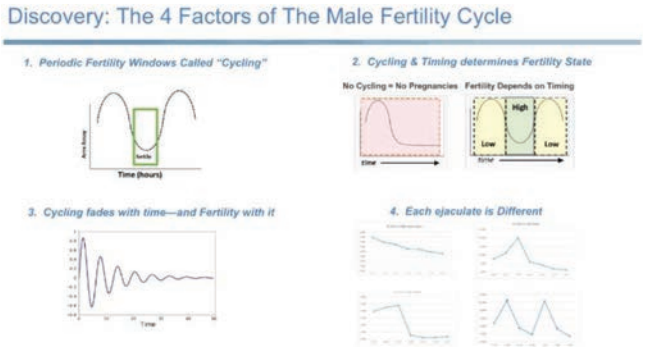
Presented By: Clifford Librach, MD

Introduction & Objective: Freshly ejaculated mammalian sperm have poor fertilizing ability; sperm must first mature. We discovered maturation occurs in sequential waves of synchronized cohorts of sperm, termed the "male fertility cycle." Our assay visualizes sperm waves by identifying molecules that become detectable during the process (e.g., Fc receptor) in ejaculated specimens. We hypothesized that male fertility cycles could predict the success of intrauterine insemination (IUI).

Methods: This was e prospective, double-blind, dual institution study of couples undergoing IUI procedures. The Arex Assay of functional Fc receptor levels (FcR) was performed on a small portion of inseminating semen samples among infertile couples undergoing IUI at two North American fertility centers. Briefly, 5 uL aliquots of semen were analyzed at 30-minute intervals to measure the proportion of sperm expressing FcR. Changes in FcR expression were observed over time. Samples were categorized as having sperm maturation waves ("cycling") or lacking them ("non-cycling,") based on FcR expression patterns. Also, the Arex Assay indicated whether sperm were in a "fertile" or "infertile" window of the male fertility cycle. Arex Assay findings were not used to alter timing of IUI procedures. Success was defined as two sequential rising hCG tests.

Results: Among n = 83 IUI cycles performed in couples at one center, 11/36 (30%) achieved pregnancies when IUI was performed in the Arex "fertile" window period and 1/47 (2%) became pregnant when IUI was done during the "infertile" window (95% Confidence Interval, CI: 0.129, 1.00; $\chi^2 = 11.12$, p = 0.0004; two-sample test for equality of proportions with Yates' continuity correction). Among n = 29 IUI cycles performed in couples at the other center, 6/15 (40%) achieved pregnancies when IUI was performed in the Arex "fertile" window and 0/14 (0%) became pregnant when IUI was done during the "infertile" window (95% CI: 0.123, 1.00; $\chi^2 = 4.834$, p = 0.0140; two-sample test for equality of proportions with Yates' continuity correction).

Conclusion: It has long been thought that the highest performing, vanguard sperm fertilize the egg. Our research suggests that sperm cooperatively work in sequential cohorts or waves, causing the fertility of an ejaculate to vary over time, as waves arise and fade in sequence. IUIs performed when a sperm wave is in the "fertile" window resulted in statistically significantly higher pregnancy rates than those performed during the "infertile" window.



Discovery: the Male Fertility Cycle in Ejaculated Sperm

Poster 32

META-ANALYSIS REVEALS ASSOCIATION BETWEEN TWO SPERM FUNCTION ASSAYS: SEMINAL OXIDATION REDUCTION POTENTIAL AND SPERM DNA FRAGMENTATION

Manesh Kumar Panner Selvam[1], Saradha Baskaran[1], Samantha O'Connell[2], Wael Almajed[1], Wayne Hellstrom[1], Suresh Sikka[1]

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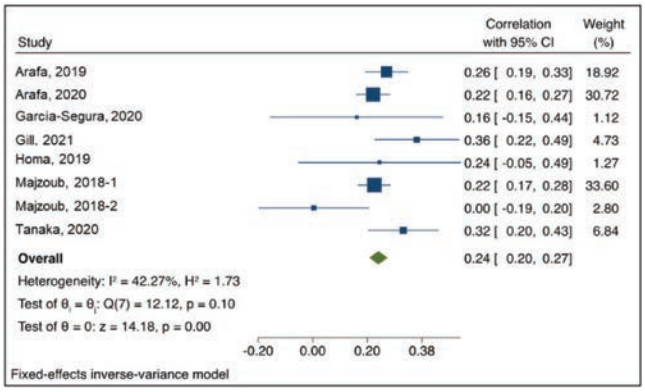
Presented By: Manesh Kumar Panner Selvam, DVM, PhD

Introduction & Objective: Seminal oxidative stress and sperm DNA damage are potential etiologies of male infertility. Oxidation reduction potential (ORP) is a novel rapid test that measures seminal oxidative stress, whereas sperm chromatin dispersion (SCD), terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL), and sperm chromatin structure assay (SCSA) are most commonly used to assess sperm DNA fragmentation (SDF). The recent WHO manual (2021) highlighted the significance of ORP and SDF assays in assessing human spermatozoa dysfunction. However, the association between ORP and SDF assays has not been fully investigated. The main objective of the present study is to evaluate the relationship between ORP and SDF by conducting a systematic review and meta-analysis of clinical studies.

Methods: A systematic literature search was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to retrieve studies related to seminal oxidative stress and SDF from databases such as PubMed, Embase, and Web of Science. The COVIDENCE (https://www.covidence.org/) tool was used to screen and identify studies evaluating both seminal ORP and SDF. The Pearson correlation coefficient between seminal ORP and SDF served as the effect size for synthesis, and the fixed-effects model was used to analyze the pooled data. The trim-and-fill method was used to evaluate publication bias among the studies included for meta-analysis.

Results: Eight studies that measured both seminal ORP and SDF of 3,491 semen samples from men attending fertility clinics were pooled in this meta-analysis. The fixed-effects model revealed that the pooled correlation coefficient (0.24; $p = 0.00$) between seminal ORP and SDF was significant (Figure 1). Furthermore, subgroup analyses indicated that the pooled correlation coefficient between ORP and SCD assays was lesser than other SDF assays, which includes TUNEL and SCSA (0.23 vs. 0.29, $p > 0.05$). There was a moderate level of heterogeneity ($I^2 = 42.27\%$) among the studies with a lack of publication bias.

Conclusion: This is the first meta-analysis to evaluate the relationship between seminal oxidative stress marker and sperm DNA damage. This meta-analysis reveals a positive correlation between seminal ORP and SDF. The present study indicates the role of oxidative stress in the development of sperm DNA damage, thus warrants exploring the clinical value of these sperm function tests in a prospective manner.



Forest plot of correlation coefficient between ORP and SDF

Poster 34

EXPANDING ACCESS TO MALE FERTILITY TESTING THROUGH VALIDATION OF AN AT HOME COLLECTION KIT

G Charles Ostermeier[1], Cristina Cardona[1], Alexander Travis[1,2]

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Presented By: G Charles Ostermeier, PhD

Introduction & Objective: G_{M1} localization patterns indicate capacitation status at the single cell level. The Cap-Score™ reports the proportion of sperm displaying G_{M1} localization patterns consistent with capacitation. Two separate studies confirmed that Cap-Score prospectively predicts a man's fertility and his probability of generating a pregnancy. TEST (TES and Tris) yolk buffer (TYB) prolongs the fertilization capacity of sperm. Here, we document that TYB enables home collection for Cap-Score.

Methods: *Validation study:* Following liquefaction, semen samples were split. Half was processed normally for Cap-Score (Control). The other half was diluted with TYB, cooled overnight and then processed the following day (Test). Paired t-tests compared the Control and Test samples.

Real-world observational study: Cap-Score and concentration were obtained from men seeking fertility assistance at reproductive endocrinology offices. Samples were either collected and processed at Clinics using the same process as the control above (Clinic) or with Home Collection kits (HC) like the Test group. Mann-Whitney tests compared the Clinic and HC samples.

Results: Cap-Score and concentration were the same for the Control and Test (33.6±1.2 vs. 34.0±1.2; $p = 0.601$; $n = 40$; 76.9±5.2 vs. 79.0±8.8; $p = 0.767$; $n = 35$ respectively).

Cap-Score was the same (29.2±0.2 vs. 29.3±0.3; $p = 0.484$) for Clinic ($n = 1889$) and HC ($n = 763$). Concentration (68.0±1.3 vs. 61.9±1.9; $p = 0.001$) was reduced with HC.

Conclusion: The validation study and real-world data demonstrated Cap-Score was consistent with HC versus immediate processing at the clinic. Reductions in concentration were anticipated with HC, as a minimum of 10×10^6 cells was originally required with processing at clinics, whereas no minimum was set for HC. Home collection would allow clinics with limited andrology staff to focus on other responsibilities. It may help to encourage men who are concerned with producing at an office or delivering samples to a clinic, to pursue fertility workups. It may also increase the availability of fertility evaluations to individuals that live far from clinics and decrease costs related to travel and time off work.

Poster 35

IN MEN SEEKING FERTILITY ASSISTANCE, DEFECTS IN SPERM CAPACITATION/FERTILIZING ABILITY ARE COMMON IN ALL AGE GROUPS, IN CONTRAST, SEMEN VOLUME AND MOTILITY DECLINED WITH AGE

Fady Sharara[1], Cristina Cardona[2], Charles Ostermeier[2], Alexander Travis[2,3]

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Presented By: Cristina Cardona, PhD

Introduction & Objective: Sperm must capacitate to become fertilization competent. Cap-Score™, which quantifies capacitation status to functionally assess male fertility, prospectively predicts pregnancy. Semen analysis (SA) does not diagnose sperm function defects and fails to predict fertility. Multiple societal factors including education, career, life goals, financial considerations, and health issues are causing couples to delay having children. Delaying parenthood raises several concerns related to reproductive success. It is generally accepted that maternal age is inversely related with fertility and pregnancy outcome. However, the influence of paternal age on male fertility parameters is largely unknown.

Objective: The objective of this study was to determine how capacitation ability, as measured by Cap-Score, and traditional semen analysis (SA) measures (Volume, Concentration, Motility) change with paternal age. The objective of this study was to determine how capacitation ability, as measured by Cap-Score, and traditional semen analysis (SA) measures (Volume, Concentration, Motility) change with paternal age.

Methods: Cap-Score and SA measures were collected from men questioning their fertility (MQF; n = 2,652; multicentric design, 35 reproductive endocrinologist (RE) prescribers, n = 16 clinics). A Mann-Whitney test was used to compare Cap-Scores between MQF and a population of men with known recent paternity (n = 76). MQF were separated into the following age groups 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, & 50+ (n = 22, 280, 926, 843, 374, 143, and 64 per group respectively). Mixed model ANOVAs were performed to evaluate associations between SA, Cap-Score, and age groups, and to account for any potential impact of Cap-Score collection kit type within the age groups (n = 763 collected at home and n = 1,889 collected at the clinic).

Results: Men questioning their fertility had reduced capacitation ability (29.2±0.15 vs. 35.3±0.88) $p < 0.001$). There was no change in Cap-Score ($p = 0.916$) or concentration ($p = 0.926$) in association with the age groups. In contrast, both semen volume ($p = 0.008$) and the percent of sperm motility ($p < 0.001$) declined with age.

Conclusion: Capacitation ability is reduced in MQF when compared to men with known paternity. In MQF and actively pursuing fertility assistance with an RE, motility and volume declined with age. Reductions in capacitation, or sperm fertilizing ability, were equally prevalent across the age groups in MQF. These data show that capacitation ability is sensitive to male fertility issues across age groups and shouldn't be reserved for older men.

Poster 37

THE CYTOLOGICAL AND VASCULAR CHANGES IN THE TESTIS FOLLOWING EXPERIMENTAL TRAUMA

Anastasiya Spaska[1], Bogdan Grytsuliak[2]

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Presented By: Anastasiya Spaska, PhD

Introduction & Objective: Among multiple factors triggering disorders of spermatogenesis, high importance possesses testicular trauma as a result of sports, domestic and industrial activity (blunt scrotal trauma, compression, rupture). In practice, they occur quite often, but their effect on testicle has not been studied enough as it is difficult to predict long-term influence on fertility. Study investigated influence of trauma on testis in a time-dependent manner.

Methods: Study completed with 32 white mature laboratory rats, divided into groups: control, day 7, day 14, day 30, day 90 following trauma. The Commission on Bioethics of the Precarpathian National University has approved the research. We developed the model of the dosed blunt testicular trauma avoiding rupture of the tunica albuginea. Microcirculatory bed of testis was examined, histologically determined diameter of convoluted seminiferous tubules, degree of damage to spermatogenic epithelium, number of cells on stage VII of development cycle, volume of Leydig cells nuclei. Electron microscopy was performed at a magnification of 4000-16,000. Statistical analysis done with the STATISTICA for Windows.

Results: On the day 7 following testicular trauma, weight of testicle and diameter of convoluted seminiferous tubules decreased, compared to control group. Tunica albuginea at the site of injury was thickened due to edema and increased connective tissue. Microcirculatory bed of injured testicle locally lost its arrangement pattern, compared to normal. Basal membrane of seminiferous tubules was thick, 19% of them expressed severe damage of spermatogenic epithelium. In 10% of the tubules only Sertoli cells and spermatogonia were present. Nucleus volume in Leydig cells decreased. On the day 30 normal structural appearance retains only in one third of seminiferous tubules. Number of cells in spermatogenic epithelium was significantly reduced. Due to atrophy of parenchyma, microvascular network around tubules was significantly deformed. With the extended time of experiment atrophic changes in parenchyma of testis were more prominent. Electron microscopy showed changes in all types of testicular cells.

Conclusion: Severe damage to spermatogenic epithelium cells may develop due to micro hematomas, disrupted blood-testis barrier with possible development of inflammation and autoimmune process. Testicular trauma in men can be complicated with hydrocele, adversely affecting spermatogenesis. Experimental data indicated that testicular trauma causes fast onset of spermatogenesis disorders, though emphasize the need for its prevention or early management to avoid inflammation and preserve fertility.

Poster 38

MOUSE ADAPTED SARS-COV-2 INDUCES HISTOPATHOLOGICAL CHANGES IN TESTIS OF LABORATORY MICE

Manesh Kumar Panner Selvam[1], Akhilesh Srivastava[1], Felix Rabito[1], Amruta Narayanappa[1], Gregory Bix[1], Xuebin Qin[2], Jay Kolls[1], Suresh Sikka[1]

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Presented By: Manesh Kumar Panner Selvam, DVM, PhD

Introduction & Objective: Testes are susceptible to SARS-CoV-2 systemic infection. Impaired spermatogenesis and leukocyte infiltration were observed in human testicular autopsy samples. Till date there is no suitable animal model available to study the testicular changes induced by SARS-CoV-2. Recently, Mouse Adapted SARS-CoV-2 (SARS-CoV-2 MA10) was reported to infect standard laboratory mice (C57BL/6) and proposed as a promising model to study multiple aspects of SARS-CoV-2 disease pathogenesis. The main objective of our study is to understand the testicular pathogenesis in C57BL/6 and immunodeficient (RAG2-/-) mice infected with SARS-CoV-2 MA10.

Methods: Reproductively mature eight weeks old C57BL/6 (n = 5) and RAG2-/- (n = 5) male mice were infected with $1x10^5$ TCID50/mouse of SARS-CoV-2 MA10 virus inoculum (50 μL) by intranasal route. Infected mice were maintained in biosafety level 3 (BSL3) facility and euthanized on day 21 post-infection. Establishment of infection was evaluated by detecting the presence of virus in lung samples using q-RT-PCR technique. Age matched uninfected mice (C57BL/6, n = 5) served as a control group. Testis from all three groups were harvested and fixed in 10% neutral formalin buffer. The fixed samples were then processed

and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

Results: Viral load was higher in lungs of RAG2-/- (8.0 ± 6.0 x10^6 copies/100 ng RNA) compared to C57BL/6 (8.6 ± 11.7 x10^5 copies/100 ng RNA) infected mice. Histological analyses of testis revealed spermatogenesis impairment in both C57BL/6 and RAG2-/- infected mice. Degeneration of germ cells was noticed with histopathological changes more prominent in the testis of RAG2-/- (Figure 1A) compared to C57BL/6 (Figure 1B) infected mice, whereas no changes were observed in uninfected mice (Figure 1C). Furthermore, detachment of Sertoli cells from the basement membrane was observed in mice infected with SARS-CoV-2 MA10.

Conclusion: The current SARS-CoV-2 mice model recapitulates the testicular pathogenesis similar to that in testis of COVID19 infected men. This model can be used to understand the pathological and molecular alterations in testis and associated male accessory sex organs caused by SARS-CoV-2 infection that may have negative impact on its reproductive potential.

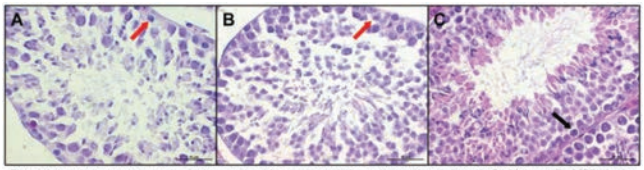

Figure 1: Impaired spermatogenesis with less number of sperm in seminiferous lumen of testis from (A) RAG2-/- and (B) C57BL/6 mice infected with SARS-CoV-2 MA10. C) testis of uninfected C57BL/6 mice. Red arrows indicate detachment of Sertoli cells from the basement membrane and black arrow indicate Sertoli cells intact to basement membrane. Scale bar = 50 µm.

Histological analyses of testicular sections

Poster 39

DISRUPTION OF A CARGO TRANSPORT SYSTEM FOR SPERM FORMATION BY A SINGLE AMINO ACID MUTATION IN MOUSE PACRG PROTEIN

Yi Tian Yap[1], Shuo Yuan[1,2], Wei Li[1], Wei Qu[1,2], Ljiljana Mladenovic-Lucas[1], Alyson Sujkowski[1], Robert Wessells[1], Jie Xu[3], Rex Hess[4], James Granneman[1], Aminata Toure[5], Jifeng Zhang[3], Zhibing Zhang[1]

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Presented By: Yi Tian Yap, BS

Introduction & Objective: A precise and coordinated interaction between specified proteins is required for correct mammalian spermatogenesis. In particular, interaction between mouse meiosis-expressed gene 1 (MEIG1) and Parkin co-regulated gene (PACRG) is essential for sperm formation and male fertility. PACRG recruits meiosis-expressed gene 1 (MEIG1) to the manchette for normal spermiogenesis, and the key amino acid on mouse MEIG1 that mediates interaction with PACRG has been identified. MEIG1/PACRG interaction is conserved in humans. The structure of the human MEIG1/PACRG complex has been resolved, and the key amino acids on human PACRG that mediate interaction with MEIG1 have also been identified and are conserved in mice. Mutations of these amino acids, particularly H121, significantly reduced MEIG1/PACRG interaction as evaluated by luciferase-based assay.

Methods: To further study the role of H121 in vivo, we mutated H121 of mPACRG using the CRISPR/cas9 system and examined the biological and physiological consequences in mice.

Results: All mutant mice were grossly normal. However, complete infertility was observed in all homozygous mutant males analyzed, accompanied with severe reduction in sperm count, while the fertility of female was not affected. Motility of the sperm cells were severely reduced, and all spermatozoa were morphologically abnormal. Histological examination of the testis demonstrates impaired spermiogenesis in mutant mice. Electron microscopy of the testes revealed severe sperm flagellar disruption in the mutant mice. Western blot analysis indicated that the protein levels of PACRG, MEIG1 and SPAG16L, a cargo protein of the MEIG1/PACRG complex, were not changed in the mutant mice. However, immunofluorescence staining revealed that MEIG1 and SPAG16L were no longer present in the manchette of the mutant sperm.

Conclusion: These findings collectively demonstrate that in vivo, H121 is a key amino acid mediating the interaction between PACRG and MEIG1 and is crucial for the downstream cargo transport that mediates sperm flagella formation. In light of the fact that MEIG1 and PACRG are conserved in humans, small molecules that interfere with MEIG1/PACRG interaction might be developed as male-based contraception drugs.

Poster 40

CHARACTERIZATION OF TSPAN33 AND LPPR3 HUMAN SPERMATOGONIAL STEM CELLS

Andre Caldeira-Brant[1], Sarah Munyoki[1], Meena Sukhwani[1], Helio Chiarini-Garcia[2], Kyle Orwig[1]

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Presented By: Andre Caldeira-Brant, PhD

Introduction & Objective: Human spermatogenesis is dependent on a population of spermatogonial stem cells (hSSC) that maintain sperm production in adult men, and therefore of great clinical importance. Most knowledge about human spermatogonia is based on nuclear morphological descriptions (A_{dark} and A_{pale}). There is scarce information about how hSSC nuclear morphology correlates with gene expression and function. We validated two novel hSSC markers, TSPAN33 and LPPR3, by associating their expression with nuclear morphology, co-expression with other spermatogonial markers, and transplantation potential.

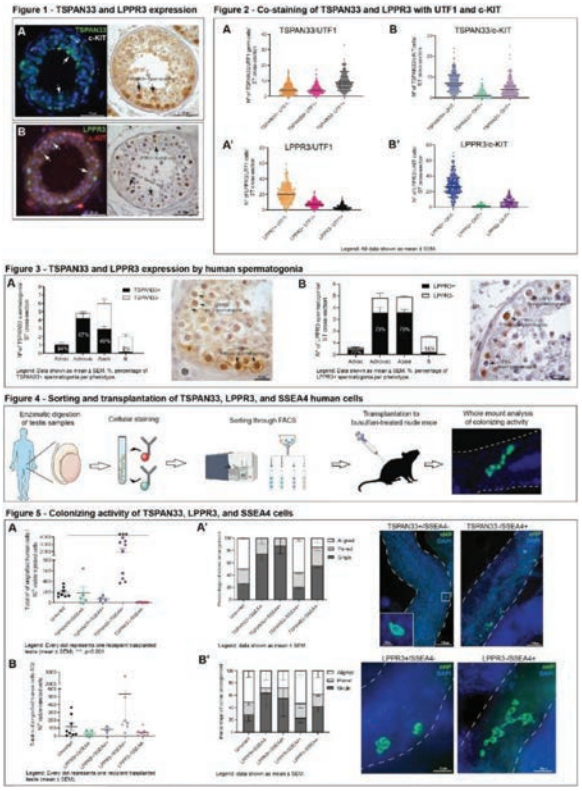
Methods: Colorimetric immunohistochemistry (IHC) was used to associate spermatogonial morphology (A_{dark} with vacuole, A_{dvac}; A_{dark} without vacuole, A_{dnovac}; A_{pale}; and B) with TSPAN33 and LPPR3 expression. Immunofluorescence co-staining was used to evaluate co-expression with markers of undifferentiated (UTF1) and differentiating spermatogonia (cKIT). Fluorescence-activated cell sorting (FACS) was used to fractionate human testis cells based on expression of TSPAN33,

LPPR3 and SSEA4. Sorted populations were tested functionally by transplantation to busulfan-treated, immuno-deficient nude mice.

Results: IHC revealed that TSPAN33 and LPPR3 were present in spermatogonia as well as other cells of the seminiferous epithelium (Fig. 1A-B). TSPAN33+ and LPPR3+ cells exhibited overlap with UTF1 (Fig. 2A) but limited overlap of expression with cKIT (Fig. 2B). TSPAN33 was expressed by most A_{dark} and half of A_{pale} spermatogonia but expression in B spermatogonia was limited (Fig. 3A). LPPR3 was expressed in ≥75% of A_{dark} and A_{pale} spermatogonia with limited expression in type B spermatogonia (Fig. 3B). After FACS and transplantation (Fig. 4) both TSPAN33+ and LPPR3+ cells engrafted recipient mouse testes (Fig.5A-B). However, while TSPAN33+ cells persisted as single and paired clones of cells (Fig. 5A'), LPPR3+ cells proliferated and produced larger clones (Fig. 5B'). In contrast, TSPAN33-/SSEA4+ and LPPR3-/SSEA4+ produced not only the greater number of donor-derived spermatogonia in recipient testes, but also the larger clone-chains (Fig. 5A-B).

Conclusion: TSPAN33 is expressed by most A_{dark} spermatogonia with decreasing expression in A_{pale} and B spermatogonia. LPPR3 is equally distributed among A_{dark} and A_{pale} spermatogonia with reduced expression in B spermatogonia. TSPAN33+ and LPPR3+ cells can engraft mouse seminiferous tubules but LPPR3+ cells have higher proliferative potential. Colonizing potential was observed in multiple subpopulations of human spermatogonia (e.g., TSPAN33+/SSEA4-, TSPAN33+/SSEA4+, TSPAN33-/SSEA4+). Future studies are necessary to better understand the activity of these subpopulations in vivo and in vitro.

Supported by NIH grant HD092084



Characterization of TSPAN33 and LPPR3 Human Spermatogonia

Poster 41

OUTCOMES OF ELEVATED ACTIVIN A ON THE GERMLINE DURING PREGNANCY AND BEYOND

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Presented By: Penny Whiley

Introduction & Objective: Male infertility and testicular cancer are understood to result from in utero testicular exposures that disrupt normal germ cell development. Activin A influences both somatic and germline cell behaviours in fetal life. Physiological perturbations during human pregnancy are often associated with high activin A levels which can occur through relatively common events such as pre-eclampsia, exposure to certain medications, or infection. Around the time of birth, testicular germ cells normally resume proliferation and transform into either the differentiating spermatogonia that initiate the first round of spermatogenesis, or into the spermatogonial stem cells (SSCs) which sustain spermatogenesis in adults. Our study objective was to investigate how elevated activin A affects the initial stages of spermatogenesis, and in particular, the formation of SSCs.

Methods: To determine whether establishment of spermatogonia or SSCs at the onset of spermatogenesis was affected, we studied a mouse model with elevated activin A bioactivity (*Inha* KO; lacks the inhibin a subunit, a potent activin inhibitor) at P0, P3, and P6. Immunofluorescent analysis of sections was used to score germ cell populations: spermatogonia (marked by SALL4+), nascent SSCs (GFRA1+) and proliferation (Ki67+). RNAseq of whole testes (n≥3/age/genotype) identified transcriptional differences. Lastly, the direct effects of activin A on primary undifferentiated murine spermatogonia were examined after 6 and 24 hours in culture by RNAsEquation (n = 4/treatment).

Results: *Inha* KO testes at P0 have 50% fewer germ cells, indicating their vulnerability to elevated activin A during fetal life. Of the remaining KO germ cells, a higher proportion are GFRA1+/Ki67+, suggesting advanced development. At P6, when the SSC population is fully established, *Inha* KO testes also have a higher proportion of GFRA1+ cells, indicating elevated activin A favours SSC formation. RNAseq confirmed transcripts associated with SSCs (*Gfra1*, *Id4*, *Etv4*, *Chd4*) and differentiated spermatogonia (*Sohlh2*, *Dnmt1*) are elevated in *Inha* KO testes. SSC cultures indicate this is an indirect effect of activin A, however SSCs do respond directly and exhibit elevated *Hox* gene expression.

Conclusion: These data demonstrate germ cells respond to activin A during fetal life, with its elevation favouring stem cell establishment, and surviving germ cells appearing developmentally advanced. The potential for human pregnancy conditions with elevated activin A to affect adult male fertility should be considered.

Poster 42

RELIABILITY AND QUALITY OF NEOVAGINOPLASTY CONTENT ACROSS YOUTUBE AND TIKTOK

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Presented By: Richard Mateo Mora, BS

Introduction & Objective: Patients, trainees, and clinicians may access social media videos, either on YouTube or TikTok. We aim to evaluate video content quality for transfeminine genital reconstruction on these platforms.

Methods: YouTube and TikTok were queried for "MTF bottom surgery" and "vaginoplasty." Videos were classified by several metrics. These included: Source: physician (urologist vs non-urologist and academic vs non-academic), patient, independent user (neither provider nor patient), other health professional; Purpose: education, entertainment, other); Presence/absence of graphics: illustrations, photos, and/or live surgery. Video reliability and quality were evaluated using DISCERN, JAMA Benchmark Criteria, and Global Quality Score metrics. Two reviewers conducted separate evaluations and their scores were averaged.

Results: Two hundred videos were reviewed, 100 on each platform, 50 for each search term. For "Male to Female (MTF) bottom surgery", YouTube had more views than TikTok (27,300 vs. 52,955, $p = 0.027$). Regarding source, YouTube had more physician sources, 46% (23/50) vs. 38% (19/50) ($p = 0.003$). Similarly, YouTube videos were more likely to have an educational purpose, and contain medical drawings, photos or operative videos. However, TikTok videos had more viewer engagement as quantified by "likes" (4180 vs. 1100, $p < 0.001$) and more followers (146,950 vs. 31,800, $p < 0.001$). Despite greater engagement, TikTok videos had lower reliability and quality scores than YouTube (23.3/40 vs. 27.9/40, $p = 0.014$) and (10.5/35 vs. 18.7/35, $p < 0.001$), respectively. Similar results were found when we searched for "vaginoplasty".

Conclusion: Despite having less physician sources, educational content, graphics, and reliability and quality scores, TikTok videos for MTF bottom surgery had more engagement via likes and followers when compared with YouTube. TikTok may represent a valuable and novel means by which to reach patients, although both platforms had a substantial amount of traffic and represent outlets for the dissemination of evidence-based information.

Table: YouTube and TikTok Search Results for "MTF Bottom Surgery"

Variable	N	Media Source		p-value
		Tik Tok (n=50)	YouTube (n=50)	
Views	49	27,300 (92,200) (182 - 3,600,000)	52,955 (281,451.8) (1239 - 5,746,246)	**0.027***
Likes	50	4,180 (11,555.8) (26 - 824,700)	1,100 (4,398.5) (0 - 44,000)	**<0.001***
Followers	50	146,950 (149,650) (223, 278,900)	31,800 (48,250) (199, 9,560,000)	**<0.001***
Subtitles				**<0.01***
No	55	9 (18.0%)	46 (92.0%)	
Yes	45	41 (82.0%)	4 (8.0%)	
Source				**0.003***
Academic (Non-Uro)	1	0	1 (2.0%)	
Academic (Urologist)	5	0	5 (10.0%)	
Ind./Physician Group (non-Uro)	34	19 (38.0%)	15 (30.0%)	
Ind./Physician Group	2	0	2 (4.0%)	
(Urologist)	11	2 (4.0%)	9 (18.0%)	
Independent User	1	0	1 (2.0%)	
Other Health Professional	46	29 (58.0%)	17 (34.0%)	
Patient				
Genre				**<0.001***
Educational	44	21 (42.0%)	23 (46.0%)	
Entertainment	19	19 (38.0%)	0	
Lived Experience	37	10 (20.0%)	27 (54.0%)	
Surgery in Video				**<0.001***
No	88	50 (100%)	38 (76.0%)	
Live	9	0	9 (18.0%)	
Animated	1	0	1 (2.0%)	
Live & Animated	1	0	1 (2.0%)	
Photos	0	0	0	
Drawings	1	0	1 (2.0%)	
Discern Reliability Score (Mean, SD)	50	23.3 (6.4) (12, 30)	27.9 (3.1) (14.5, 35)	**0.014***
Discern Quality Score (Mean, SD)	50	10.5 (3.4) (7, 19.5)	18.7 (5.5) (5, 29.5)	**<0.001***

Numbers represent median (IQR) (min – max) for continuous variables and frequency (column percent) for categorical
*Significant at p=0.05 (Wilcoxon rank sum or Pearson's χ^2/Fisher's Exact)

YouTube and TikTok Search Results for "MTF Bottom Surgery"

Poster 43

SYSTEMATIC REVIEW: THE NEOVAGINAL MICROBIOME

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Presented By: Richard Mateo Mora, BS

Introduction & Objective: The natal vaginal microbiome has been well-established and is known to protect against pathogenic microorganisms. More series examining the microbiome of the neovagina created after gender affirming surgery (GAS) are emerging. We sought to review neovaginal colonization and inflammatory patterns, and factors that may impact this.

Methods: A systematic review was conducted in concordance with PRISMA guidelines. PubMed, Embase, Ovid, World of Science, and Cochrane were searched for the keywords "transgender vagina microbiome," "neovagina microbiome," "gender affirming surgery

microbiome," "transgender women vaginal flora," and "neovagina flora." Inclusion criteria were relevant prospective or retrospective cohort studies, case control studies, and randomized trials. Abstracts and case reports were included they contained adequate information. Letters to the editor, book chapters, and articles not in English were excluded.

Results: Thirteen articles were included, totaling 458 patients. Neovaginal construction was most commonly performed with penile and scrotal skin grafts, sigmoid segments, and peritoneal grafts. The neovaginal microflora identified were generally polymicrobial and shared similarities with the native tissue. Nine studies identified *Lactobacillus*: 5/6 for penile skin, 1/3 for sigmoid, 1/1 for peritoneum, and 2/3 for other graft types, suggesting that the neovagina may support *Lactobacillus* either innately, via rectal migration or oral probiotic supplementation. A polymicrobial, bacterial vaginosis-like environment was found in nine studies. Inflammatory markers were also described: 2/6 for penile skin, 2/3 for sigmoid, 0/1 for peritoneum, and 1/3 for other graft types. Scant data were available on the impact of time since surgery, oral hormones, dilating, sexual practices, or douching on the neovaginal microbiome.

Conclusion: Understanding the polymicrobial microenvironment of the neovagina is important for clinicians as this may predispose patients to inflammation, pain, and infectious outcomes. Future research should focus on standardizing testing, classification systems, evaluating, and treating neovaginal dysbiosis.

Poster 44

CHARACTERISTICS OF SYSTEMIC ANDROGEN THERAPY FOR HYPOACTIVE SEXUAL DESIRE DISORDER IN FEMALES - A CLAIMS DATABASE ANALYSIS

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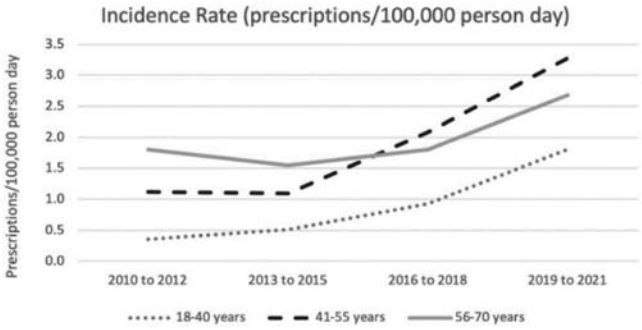
Presented By: Pranjal Agrawal, BA

Introduction & Objective: Hypoactive sexual desire disorder (HSDD) is a rarely diagnosed sexual dysfunction though it is estimated to impact 10% of adult women. Androgen therapy (with or without estrogen) has been shown to improve libido in women with HSSD; however, because of limited research of women with HSSD and concerns for adverse effects following testosterone therapy utilization has been limited. Our objective was to use a large US claims database to describe prescribing trends in systemic testosterone therapy for HSSD.

Methods: A US health research network (the TriNetX Diamond Network) of over 190 million patients, encompassing prescriptions and healthcare encounters, was queried from 2010 to September 2021. Adult female patients with HSDD were identified (ICD-10 F52.0) and testosterone prescription trends, route of administration, and coadministration with estrogen were extracted. Significance of incidence over time [prescriptions/person-day] was assessed in 2010-2012, 2013-2015, 2016-2018, and 2019-2021 using Chi Square for trend. p values less than 0.05 were considered significant.

Results: 37,491 women diagnosed with HSDD were identified with a mean age of 46.5±12.4 years, out of which only 3.9% (n = 1444) were prescribed systemic testosterone (mean age 50.6±10.0 years). Between 2010 to 2021, a statistically significant increase in testosterone prescriptions for HSDD was observed for women in the age group of 18-40 years and those in the age group of 41-55 years. (Figure 1) 56.8 % prescriptions were for injectable testosterone, 29.3% for topical, and 13.9% with the route of administration unknown. Five hundred fifty-six women received just one prescription for systemic testosterone, 327 received 2-3 prescriptions, 262 received 4-9 prescriptions, and 299 received 10+ prescriptions. Five hundred forty-three women were co-prescribed systemic estrogen, and 259 women were co-prescribed vaginal estrogen; 642 women did not receive any estrogen prescription.

Conclusion: Though systemic testosterone therapy for women with HSDD remains rare and poorly researched, its use has increased since 2010. Its duration, route, and co-administration with estrogen remains highly variable, and long-term compliance with systemic testosterone therapy remains low. Additional studies are needed to support the strong evidence of androgen therapy in effectively treating women with sexual health concerns.



Testosterone Prescriptions for HSDD from 2010 to 2021

Poster 45

CACHEXIA MANAGEMENT WITH TESTOSTERONE REPLACEMENT THERAPY: A CONTEMPORARY REVIEW OF THE LITERATURE

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Presented By: Armaan Singh, BA

Introduction & Objective: Prevalence of adult-onset hypogonadism (AOH) is increasing and is currently estimated to be 6-12% in the general population. AOH is often associated with cachexia, a progressively debilitating loss of skeletal muscle often seen in late-stage chronic medical diseases such as malignancies, chronic obstructive pulmonary disease (COPD), and HIV. Cachexia contributes to mortality and morbidity with reduced quality-of-life (QoL) and disease treatment options.

This comprehensive review highlights Testosterone Replacement Therapy (TRT) for management of AOH in patients with cachexia, with an emphasis on clinical outcomes and side effects.

Methods: A comprehensive PubMed literature review was performed to identify articles published between 2000-2021 on TRT and cachexia-related chronic medical diseases such as malignancies, COPD, and HIV. Search terms included 'TRT', 'cachexia', and 'muscle wasting'. Relevant English articles were included.

Results: Ten studies were included in our review out of 364 initial search results. In three studies of TRT in cancer patients, there were mixed results on the effects of TRT on QoL assessment (Table 1). While one study reported an improvement in QoL and lean body mass with adjunct testosterone, two others reported no improvement of QoL from testosterone therapy. Of note, one of the two studies that reported no improvement of QoL saw no significant increase in total testosterone levels. Across three studies of TRT in COPD patients, there was consistent improvement in exercise capacity and disease condition with TRT, suggesting that TRT is a promising therapy for improving patient condition. Lastly, across four studies of TRT in HIV patients, TRT resulted in notable improvement in body weight, muscle mass, function and QoL in HIV-infected men.

In the studies reviewed, there were minimal serious adverse events reported. Some include grade 3 liver injury and cases of pneumonia in the testosterone-treated group among studies of cancer patients, although these events occurred in only a small number of patients per cohort.

Conclusion: Based on recent studies, there is robust data for the clinical benefits of TRT for the management of certain subgroups of cachexic patients, specifically those with COPD and HIV. Furthermore, TRT is well established with low toxicity in male hypogonadism patients. Because there are mixed results in cancer cachexia patients, further investigation of its long-term efficacy is warranted.

Table 1. Breakdown of literature review findings, by study

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EXOGENOUS LEPTIN TREATMENT ALTERS THE TESTICULAR IMMUNE MICROIMMUNE-ENVIRONMENT

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Presented By: Alexandra Dullea, MS

Introduction & Objective: In males, Leydig cells (LCs) are the primary source of testosterone (T) production. Dysfunction of the LC can lead to T deficiency and male hypogonadism. The development of LCs is influenced by the paracrine factors released by the testicular microenvironment (TME). Leptin significantly influences hormonal regulation and is produced in adipose tissue. In our recent study, we demonstrated that leptin, a paracrine factor secreted by TME, is critical for Leydig stem cell (LSCs) differentiation into Adult Leydig Cells (ADL), and subsequent T production. However, we lack an in-depth understanding of the effect of leptin on the testicular immune microenvironment. This study evaluates the effect of different doses of leptin on the testicular immune microenvironment.

Methods: We selected a murine model (C57/BL6 mice purchased from Jackson Laboratory) to conduct the study. Mice (n = 5 per group) were subjected to intraperitoneal leptin injections at concentrations of 0 mg (control), 10 microgram or 100 micrograms. Mice were injected with leptin every day for 10 days. After treatment, mice were euthanized, and blood was collected from the tail and was processed for complete blood count (CBC) profiling and hormonal (T, LH, and FSH) profiling.

Results: The CBC profiling data was notable for a significant difference between the control and leptin 10ug in the number of neutrophils, lymphocytes, monocytes, and platelets. The control group had a significantly higher concentration of these cell types compared to the leptin 10ug group ($p = 0.05$). Interestingly, when comparing control to the 100ug group, the only significantly different blood values were monocytes and eosinophils, with a higher concentration present in the 100ug group. While there was no significant difference in the amount of red blood cells between the groups, mean corpuscular hemoglobin concentration (MCHC) was significantly altered with increased in both the 10ug and 100ug groups. The hormonal data showed that LH was significantly elevated in leptin 10ug group when compared to control ($p = 0.05$).

Conclusion: The findings suggest that low dose of leptin have a significant impact not only on the levels of LH, but importantly also on the markers of immune microenvironment. Therefore, future studies will focus on exploring the differential impact that low doses of leptin might have on several of the immune markers in testicular microenvironment.

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TAMOXIFEN ALTERS TESTICULAR STEROIDOGENESIS AND SPERMATOGENESIS VIA AN OXIDATIVE STRESS-MEDIATED SIGNALING: POSSIBLE ROLE OF CESSATION

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Presented By: Roland Akhigbe, MBBS

Introduction & Objective: Tamoxifen is a non-steroidal anti-estrogen that is used in the management of estrogen-dependent breast cancer. As an endocrine modulating drug, it has been speculated to influence testicular function; however, reports on its impact on male fertility are scarce. Hence, we investigated the effect of tamoxifen and its cessation on male sexual behavior, steroidogenesis and spermatogenesis. The roles of oxidative stress, inflammation, and apoptosis were also explored.

Methods: Forty adult male Wistar rats were randomly allotted into four groups (n = 10/group), vehicle-treated, tamoxifen-treated, vehicle-treated + reversal, tamoxifen-treated + reversal. Administration was once daily via gavage and lasted for 4 weeks. The dose of tamoxifen used was the Human Equivalent Dose for rats.

Results: Tamoxifen significantly impaired penile reflexes and male sexual behaviour. Also, tamoxifen markedly increased testicular injury markers (gamma glutamyl transferase, lactate dehydrogenase) and reduced sorbitol dehydrogenase activity. In addition, tamoxifen was observed to reduce circulatory concentrations of dopamine, GnRH, FSH, LH, testosterone and estrogen and testicular testosterone, 3β-HSD and 17β-HSD. Furthermore, tamoxifen reduced sperm count, motility and viability, but increased the percentage of abnormal sperm morphology. The observed tamoxifen-induced alterations were in tandem with testicular histopathological findings (shrunken seminiferous tubules, widened interstitial space, and reduced luminal spermatozoa, epithelial height and markers of spermatogenic index) and accompanied by a rise in markers of testicular oxidative stress (uric acid, xanthine oxidase, malondialdehyde), inflammation (myeloperoxidase activity, TNF-α, IL-6), and apoptosis (caspase 3). This was associated with reduced testicular antioxidants (glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase). These tamoxifen-driven perturbations were reversed by tamoxifen cessation.

Conclusion: This study revealed that tamoxifen impairs male sexual function, testicular steroidogenesis and spermatogenesis by suppressing the hypothalamic-pituitary-testicular axis via an oxidative stress-sensitive signaling.

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EFFECTS OF ENDOCRINE DISRUPTING CHEMICALS, ACETAMINOPHEN, AND NSAIDS ON IMMATURE SERTOLI CELL FUNCTIONS

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Presented By: Maia Corpuz, BS, MS

Introduction & Objective: Perinatal exposure to acetaminophen (AC), non-steroidal anti-inflammatory drugs (NSAIDs), and endocrine disrupting chemicals (EDCs) may contribute to male infertility. Hospitalized Infants may be exposed to endocrine disrupting chemicals (EDCs) such as di-(2-ethylhexyl) phthalate (DEHP) or genistein, a phytoestrogen abundant in soy-based formula. Meanwhile, feverish infants may be treated with AC or NSAID ibuprofen (IB). Considering the essential role of Sertoli cells in germ cell development and function, disruptive effects of EDCs or drugs on immature Sertoli cells could jeopardize spermatogenesis and contribute to male infertility. Thus, our goal is to examine the effects AC/NSAIDs and EDCs pose to immature Sertoli cells, and identify the functional pathways they target.

Methods: Immature TM4 mouse Sertoli cells were used as model to assess the effects of treatments with AC, IB, GEN, or the DEHP bioactive metabolite mono-(2-ethylhexyl) phthalate (MEHP), alone or mixed at 10, 50, and 100μM, for 24-72 h on cell viability and proliferation using MTT assays. The effects of AC and GEN on gene expression were further examined by qPCR analysis.

Results: AC $\geq$ 50μM and IB and GEN at 100μM alone, but not MEHP, significantly decreased cell viability/proliferation, while mixtures had similar effects to single compounds. The Sertoli cell marker anti-mullerian hormone (*Amh*) was significantly upregulated by 50μM AC and GEN in mixture but not alone, suggesting a synergistic effect at that concentration. In contrast, *Sox9* expression was significantly decreased by all treatments. GEN $\geq$ 10μM and AC-GEN mixtures significantly downregulated *Cox-1* and *Cox-2* expression, while AC alone did not affect *Cox1* but reduced *Cox2* at 50μM. Prostaglandin D synthase *Ptgds* was upregulated by GEN $\geq$ 10μM, AC 50μM and their mixtures, while Prostaglandin E synthase *Ptges* showed downregulated trends with 100μM treatments, suggesting differential effects of the compounds on these prostaglandins.

Conclusion: Cell viability and gene expression analysis suggest that exposures to AC/NSAIDs and GEN-MEHP alone and mixed dysregulate immature Sertoli cell development. Elucidating the molecular mechanisms mediating these effects may contribute to a better understanding of the origins of male infertility.

Poster 51

CAMP AND ADENOSINE ARE MOLECULAR MEDIATORS THAT CONTROL NHE3-DEPENDENT LUMINAL ACIDIFICATION IN THE EPIDIDYMAL LUMEN

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Presented By: Larissa Berloffa Belardin, PhD

Introduction & Objective: The epididymis creates an optimal acidic luminal environment in which spermatozoa mature and are stored in a dormant state. Proton secretion is achieved by the proton pump, V-ATPase, located in clear cells (CCs), and by the sodium-proton exchanger, NHE3, located in principal cells (PCs). PCs also secrete bicarbonate via CFTR, a process that would induce luminal alkalization, preparing sperm for ejaculation. In addition, PCs secrete ATP, which is then hydrolyzed into adenosine by ectonucleotidases. As a first step to explore the factors that control the dual role of PCs (acid vs base secretors), we examined here the respective roles of cAMP and adenosine

in the subcellular localization of NHE3 in epididymal PCs perfused at different luminal pH.

Methods: Epididymis of C57Bl/6Ncrl mice were perfused in vivo at pH 6.0 or 7.8. The effect of cpt-cAMP (1 mM), a cAMP permeant analog, was examined at alkaline pH (pH 7.8). The effect of adenosine (100 μm) was examined at acidic luminal pH (pH 6.0). Electron microscopy was used to quantify the length of PC stereocilia. High-resolution confocal microscopy was used in combination with brightfield imaging to visualize NHE3 location in PC stereocilia.

Results: Electron microscopy showed longer stereocilia in PCs perfused at pH 7.8 versus 6.0. This was accompanied by an increase in the amount of NHE3 present in stereocilia ($p < 0.0001$). By contrast, at pH 6.0, NHE3 was mainly located in the sup-apical pole of PCs. cpt-cAMP prevented alkaline pH-induced accumulation of NHE3 in stereocilia ($p < 0.0001$). Addition of adenosine induced the accumulation of NHE3 in PC stereocilia compared to pH 6 solution without adenosine ($p < 0.001$). We showed that PCs express the purinergic A3 receptor, which induces a reduction of intracellular cAMP when activated by adenosine. We are currently exploring the potential role of this receptor in the adenosine response of PCs.

Conclusion: Our results show that PCs can detect changes in luminal pH and adapt by modulating the amount of NHE3 that is located at their surface. In other organs, cAMP activates CFTR and inhibits NHE3. We propose that reduction of intracellular cAMP induced by luminal adenosine, and elevation of intracellular cAMP concentration secondary to basolateral stimulation contribute to the switch of PCs from NHE3-dependent proton secretion to CFTR-dependent bicarbonate secretion.

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CHARACTERIZATION OF ATP RELEASE BY PRINCIPAL CELLS IN THE EPIDIDYMIS

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Presented By: Kéliane Brochu, BS

Introduction & Objective: We previously showed that ATP, secreted from epididymal principal cells (PCs), is part of an extracellular communication network that leads to activation of neighboring clear cells (CCs). ATP and its hydrolysis product adenosine stimulate V-ATPase-dependent proton secretion by CCs. Luminal acidification maintains sperm in a quiescent state during their transit in the epididymis. We reported activation of ectonucleoside triphosphate diphosphohydrolases (ENTPD) which hydrolyze ATP at alkaline pH (Battistone et al. 2019). Here we dissected the molecular mechanisms that participate in ATP secretion in PCs.

Methods: The immortalized epididymal principal cell line (DC2) was cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with DHT at 33°C. ATP release was measured using a luciferin–luciferase based ATP bioluminescence assay. A pharmacological approach and RT-PCR were used to identify transporters and regulators of ATP secretion in DC2 cells.

Results: Constitutive ATP secretion by DC2 cells was observed, which was significantly inhibited by the Pannexin-1 (PANX-1) inhibitor, carbenoxolone (CBX,10μM), and we confirmed the participation of CFTR in ATP secretion (Ruan et al. 2012). In addition, we used the ENTPD inhibitor, polyoxotungstate-1 (POM-1, 10μM), to characterize extracellular ATP hydrolysis. Unexpectedly, we found almost no ATP in the culture medium of DC2 cells treated with POM-1. This result indicates that ATP degradation by ENTPD in DC2 cells is low under the experimental conditions used, and that POM-1 inhibited ATP secretion. POM-1 can also inhibit the purinergic receptor P2X7, an ATP-gated cation channel. RT-PCR showed high expression of P2X7 in DC2 cells as well as in mouse caput epididymidis. Thus, our results indicate the role for P2X7 in the basal release of ATP.

Conclusion: Our study highlights the participation of PANX-1 in ATP release and its regulation by CFTR. Our results also uncover the unexpected role for P2X7 in ATP signalling in the epididymis. P2X7 is well-known for its pro-inflammatory role through activation of the NLRP3 inflammasome. However, accumulating evidence suggests that P2X7 is also active in health. Interestingly, ATP release through the synergistic action of P2X7 and PANX-1 has been reported in several tissues. We are currently exploring the respective role of P2X7 and PANX-1 in ATP signalling under physiological and pathophysiological conditions in the epididymis.

Poster 53

NOVEL MECHANISMS OF HOST DEFENSE IN THE EPIDIDYMIS

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Presented By: Caitlyn Myers, BS

Introduction & Objective: The epididymis plays a critical role in protecting sperm from invading pathogens that can ascend the male tract, causing inflammation and infertility for years following an infection. The epididymis relies heavily on antimicrobial proteins (AMPs) to defend against these pathogens, but it remains unclear mechanistically how these AMPs function. We previously established a nonpathological, functional amyloid is in the epididymal lumen and contains the amyloid forms of four CRES subgroup members (CRES, CRES2, CRES3, cystatin E2), a reproductive subgroup within the family 2 cystatins of cysteine protease inhibitors. Further, the epididymal amyloid matures along the length of the tubule transitioning from a matrix in the caput to fibrils in the cauda. Our studies show that various maturational states of CRES amyloids and the epididymal amyloids exhibit potent antimicrobial activity against multiple uropathogenic bacterial strains including *E. coli*, *S. aureus*, and *N. gonorrhoeae*, suggesting they play important roles in host defense. We hypothesize that CRES amyloids and epididymal amyloids use multiple mechanisms to protect against pathogens

including trapping of bacteria and permeabilization of bacterial membranes.

Methods: Negative-stain transmission electron microscopy (TEM) was used to visualize interactions between amyloid and bacteria, while a Live/Dead assay was performed to determine if the amyloids permeabilize bacterial membranes. Colony forming unit (CFU) assays were used to validate the number of bacteria in each experiment.

Results: TEM showed that both CRES amyloids and epididymal amyloids surround and trap bacteria. Further, the Live/Dead assay revealed that bacterial membranes were permeabilized by the amyloids in both a strain-dependent and a structure-dependent manner. This was confirmed by TEM which showed bacteria with ruffled membranes or those that had transitioned into bacterial ghosts (bacteria that have extruded their cytosol and DNA yet still retain an intact and functional membrane). Interestingly, CRES monomer and the epididymal amyloids were also shown to transition to higher-ordered amyloids following incubation with bacteria. These observations suggest that CRES and the epididymal amyloids are dynamic structures that can assemble off bacterial membranes.

Conclusion: Taken together, our results show that CRES amyloids and the epididymal amyloids use multiple antimicrobial mechanisms to defend against pathogens including trapping, membrane permeabilization, and induction of bacterial ghosts. In addition, the presence of pathogens induces structural changes in CRES and the epididymal amyloids, contributing to a rapid host defense response.

Poster 56

"SPERM EXTRAGENOMIC CONTRIBUTIONS IN PREGNANCY LOSS AND CONGENITAL MALFORMATIONS: A CASE CONTROL STUDY"

Vidhu Dhawan, Manoj Kumar, Neena Malhotra, Neeta Singh, Vatsla Dadhwal, Rima Dada

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Presented By: Vidhu Dhawan, MBBS, MD

Introduction & Objective: Unravelling the various underlying etiologies of recurrent pregnancy loss (RPL) has always been an enigma. The incidence of birth defects is higher in pregnancies which culminate in stillbirths and spontaneous abortions. The contribution of sperm molecular factors in the evaluation of RPL, have always been less investigated. The complex interplay of sperm genomic and extragenomic contributions is being evaluated as an indicator of successful pregnancy outcomes.

Methods: Semen sample was obtained from male partners of couples experiencing recurrent pregnancy loss (RPL) (n = 100), and those who reported conception with previous unexplained congenital malformations (CMF) (n = 25) patients and healthy fertile controls (n = 75). q-PCR analysis was done by $2^{-\Delta\Delta Ct}$ method for the relative quantification of *FOXG1*, *SOX3*, *STAT4*, *RPS6*, *RBM9*, *RPL10A*, *RPS17*, *RPL29*, *WNT5A*, *HSP90*, *TOMM7*, *EIF5A*, *OGG1 and PARP1* after normalization to *GAPDH* and *β-actin*. The levels of seminal ROS (RLU/sec/million sperm), DNA damage (%) and relative sperm telomere length (STL) were assessed by chemiluminescence and sperm chromatin structure assay (SCSA) and qPCR respectively.

Results: The relative expression of *FOXG1*, *SOX3*, *RPS6*, *RBM9*, *HSP90*, *TOMM7* and *OGG1* was found to differ significantly between RPL patients and controls, while expression of *HSP90* and *TOMM7* differed significantly in CMF patients. The median ROS level was seen to be higher (> 28) in RPL [41.6 (4.5-567.41)] and CMF patients [44.8 (7.9-562.9)] w.r.t controls [17.9(2.5-43.90] ($p < 0.001^{***}$). The mean DFI levels were seen to be higher (> 31) in RPL (33.8 ± 6.3) and CMF patients (35.14 ± 6.01) w.r.t controls (25.8 ± 4.02) ($p < 0.001^{***}$). The mean STL in RPL and CMF group showed a significant negative correlation with both ROS and DFI levels ($p < 0.001^{***}$).

Conclusion: The paternal contributions in the successful clinical pregnancy outcomes has largely remained in a flux. The assessment of the expression profile of the complex repertoire of the retained sperm RNA may be integrated as the part of seminal biomarkers.

Poster 57

SPERM INCUBATION CONDITIONS INFLUENCE THE SUCCESS OF PRE-IMPLANTATION EMBRYO DEVELOPMENT

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Presented By: Darya Tourzani, BS

Introduction & Objective: In the field of assisted reproductive technologies (ARTs), successful pregnancy outcomes are highly dependent on several factors including the production of a high-quality embryo and successful implantation. It has been established that at the moment of fertilization the sperm contributes PLCζ (male factor that triggers egg activation) and the paternal DNA. However, in the last few years, the contribution of the sperm past fertilization has been correlated to successful embryo development. Our lab has shown that when sperm are pre-incubated prior to in vitro fertilization in conditions that lack energy resources, glucose and pyruvate, fertilization success can be significantly improved and recovered in some mouse models with a sub-sterile/sterile phenotype. This sperm treatment, known as Sperm Energy-restriction and Recovery (SER), consists in the removal of energy resources from the media followed by the restoration of energy substrates. In addition to those improvements in fertilization, improvements were seen in embryo development and implantation success leading to full-term pregnancy. However, the early events of fertilization altered by the SER treatment remained unclear. Therefore, the aim of our work was to investigate how changes in the sperm pre-incubation conditions can dictate those pre-implantation events leading up to the development of a successful embryos.

Methods: In order to address this, we compared the results of the SER treatment on C57BL/6J sperm for in vitro fertilization. Our goals were to compared different post-fertilization events and pre-implantation embryos by methods of advanced microscopy.

Results: Our results indicated that the SER treatment does not alter the rate of sperm penetration and sperm head decondensation. However, SER-generated embryos result in advanced timing in PN-stage formation without compromising pre-implantation cleavage rates compared to their control-generated embryo counterparts. We further analyzed pre-implantation embryos at the blastocyst stage by assessing cell number, size, and inner cell mass (ICM)/trophectoderm (TE) cell ratio. We found that SER-generated embryos are bigger with a small increase in cell numbers, although the ICM/TE ratio is maintained.

Conclusion: Altogether these data suggest that the sperm incubation conditions can influence the success of pre-implantation embryos and that the SER treatment not only improves in vitro fertilization but increase the possibility of obtaining a high-quality pre-implantation embryo.

Poster 58

DEVELOPMENT OF A NOVEL METHOD FOR ASSESSING CLINICAL EFFICACY OF AN ON-DEMAND MALE CONTRACEPTIVE

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Presented By: Carla Ritagliati, BS, PhD

Introduction & Objective: Mammalian sperm are stored in the epididymis in a dormant state; they are immotile and unable to fertilize the oocyte. After ejaculation, in the female reproductive tract, sperm begin swimming and initiate a process called capacitation, where they acquire hyperactivated motility and acrosomal responsiveness, becoming competent to fertilize.

An initial event in capacitation and hyperactivation is the HCO_3^-induced sAC-mediated increase in cAMP level. The phenotypes of sAC KO mice and men suggest a strategy to safely and effectively deliver a sAC inhibitor to men. Two independently generated sAC KO mouse strains and humans homozygous for sAC disrupting mutations exhibit male-specific sterility. In both mice and men, sAC KO sperm are immotile, which can be rescued by cAMP analogs, and very importantly, they exhibit no other overt phenotypes. Systemic exposure of sAC inhibitors acutely inhibits male fertility. One hour after injecting male mice with one of the most potent inhibitors, their spermatozoa are immotile, and the males have severely reduced fertility. The effect is reversible; motility returns to normal by the next day (see Balbach poster/oral presentation).

The aim of this work was to develop methods for measuring efficacy of sAC inhibitors on human spermatozoa functions to be used during clinical trials.

Methods: The most promising sAC inhibitors in vitro dose responses were tested by measuring cAMP accumulation, PKA substrate phosphorylation, motility/hyperactivation and induced acrosome reaction in human spermatozoa. The chosen appropriate concentrations were assayed in motility dilution experiments.

Results: Due to its tight binding kinetics, the best compounds inhibit essential functions in human spermatozoa, which withstand the dilution effects presumably encountered when ejaculated sperm enter the inhibitor-free female reproductive tract.

Conclusion: This methodology for assessing sAC inhibitors on human spermatozoa motility of ejaculated sperm can be leveraged as a pharmacodynamic endpoint during Phase 1/2a clinical trials. The ability to indirectly test efficacy, by visualizing the effects on sperm functions essential for fertilization during the early phases of clinical testing, defines on-demand contraceptives as relatively unique among potential therapeutics. By assessing efficacy of the compound following dilution into inhibitor-free media, analogous to the dilution that will normally occur following ejaculation into the vagina, we will be able to narrow in on a dosage range that should provide contraceptive efficacy during Phase 2b/3.

Poster 59

DETECTION OF SEMINIFEROUS TUBULE HARBORING SPERMATOZOA BY OPTICAL COHERENCE TOMOGRAPHY IN TESTES OF NON-OBSTRUCTIVE AZOOSPERMIA RAT MODEL

Yanhe Lue[1], Luyang Yu[2], Junze Liu[2], Hang Yang[2], Hyle Park[2], WonHo Kim[1], Youngju Pak[1], Jacob Rajfer[3], Christina Wang[1], Ronald Swerdloff[1]
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Presented By: Yanhe Lue, MD

Introduction & Objective: Men with infertility due to non-obstructive azoospermia (NOA) have no sperm in their ejaculate, yet small pockets of sperm often exist in some of the seminiferous tubules. Microsurgical testicular sperm extraction (micro-TESE) is the surgical procedure performed to identify and extract occult sperm for ICSI and IVF. Although successful sperm retrieval rates reported for micro-TESE are as high as 50%, the micro-TESE procedure remains a challenge for surgeons to efficiently locate these cryptic sperm based solely on visual cues under the operating microscope. Optical coherence tomography (OCT) is ideal for visualizing sperm through the wall of tubules because of its capability for micron-scale resolution at about 2 mm beneath the surface of biological tissue.

Methods: We have developed a customized OCT system and utilized this system in a rat NOA model. NOA was induced in 15 rats by intraperitoneal injection of busulfan (10 mg/kg) on day 1 and day 22 to eliminate differentiating germ cells while maintaining spermatogonial stem cells (SSCs). Two to three months after the last injection, some of the residual SSCs re-initiate spermatogenesis and produce spermatozoa in a few seminiferous tubules mimicking NOA in men. Briefly, rat

testes were harvested and six small incisions were made in the tunica albuginea of each testis to expose tubules. We performed OCT scanning on 180 spots from 30 NOA testes. After OCT scanning and image acquisition, we sniped tubules from each scanned site to determine under the microscope whether the OCT visualized tubules contained spermatozoa. The remaining testis tissues were prepared for histological examination.

Results: The time of acquiring the data of one image was 25 seconds, and processing the quick En Face image was about 100 seconds. Comparing OCT results with those from microscopic examination, demonstrated 95% accuracy of the OCT method to locate tubules with spermatozoa; the sensitivity was 96.5%, specificity was 92.4%; the positive predictive value was 95.7% and the negative predictive value 93.9%.

Conclusion: Our customized OCT system is an excellent tool to locate seminiferous tubule harboring spermatozoa in rat NOA testes. We plan to use a modified OCT system on *ex-vivo* human seminiferous tubules followed by in vivo examination of human NOA testes. This OCT system will assist surgeons treating infertile men with NOA who wish to father children.

Poster 60

SPERMCHECK FOR USER-MONITORING OF SPERM SUPPRESSION IN A HORMONAL MALE CONTRACEPTIVE TRIAL

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Presented By: Yanhe Lue, MD

Introduction & Objective: Hormonal male contraception is achieved by administering an exogenous androgen alone or in combination with a progestin to suppress spermatogenesis creating reversible azoospermia or severe oligozoospermia in the ejaculate. We aimed to validate use of the SpermCheck Vasectomy (SpermCheck-V) home immunodiagnostic kit by participants in a hormonal male contraceptive trial to monitor suppression of sperm concentration at a level that is consistent with highly effective contraceptive efficacy.

Methods: This is a prospective sub-study of the ongoing Phase 2b multi-center clinical trial: "Study of Daily Application of Nestorone® (NES) and Testosterone (T) Combination Gel for Male Contraception." in a single center. We invited couples to participate in the sub-study and provided semen samples. The participants were asked to process a small portion of their own semen sample using the SpermCheck-V kit under direct observation of the laboratory technologist. We compared positive/negative SpermCheck-V results performed by participants in the clinic against hemocytometer-derived sperm concentrations assessed by an andrology laboratory technologist. Trial participants were asked to complete a 10-item survey and queries on the user's perceived ease or difficulty understanding how to perform, read, and interpret the SpermCheck-V test and results

Results: By examining 188 semen samples from 35 participants, SpermCheck-V returned a negative result in 100% of hemocytometer-derived azoospermic samples (n = 103), and 95% of samples with sperm concentrations between 0.01 to 0.2 million/mL (n = 20). Two samples with sperm concentration close to the kit's published threshold of 0.25 million/mL (0.21 and 0.23 million/mL) produced marginally positive results. Using a positive result threshold of > 0.2 million/mL, the accuracy was 99.5 %, sensitivity 100%, specificity 99.2%, Positive Predictive Value 98.5%, and Negative Predictive Value 100%. Trial participants noted the overall ease of the SpermCheck-V, with respect to semen sample preparation, test timing, and result interpretation.

Conclusion: SpermCheck-V is an excellent device for monitoring spermatogenesis suppression in hormonal male contraceptive trials. Participants performed SpermCheck-V with 99.5% accuracy and correctly diagnosed sperm concentration of > 0.2 million/ml in 100% of samples. The participants expressed confidence in their ability to accurately use this test at home without difficulty. As an at-home test kit, SpermCheck-V can minimize the need for return visits to monitor for suppression of spermatogenesis and maintenance of suppression, increasing the potentially practicality of hormonal male contraception.

Poster 61

COMPARATIVE ACCURACY OF THE NOVEL SPERMAN® SPERM SELECTION TECHNOLOGY VERSUS SWIM UP METHOD

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Presented By: Burak Özkösem, PhD

Introduction & Objective: One (1) out of every 5 couples in the world has difficulty in conceiving, and most of the couples start having children at a later age due to socioeconomic reasons. According to the World Health Organization's (WHO) report in 2017, sperm count in men has decreased by 59% since 1973. Infertility treatment is a financial and emotional burden in most countries. Moreover, the success rate of IVF treatments is around 30%, and half of this is due to the inability to accurately measure the sperm quality of the man and the inability to select the healthiest sperm cells. Therefore, choosing the right sperm cell in IVF treatments is the first condition for successful results. But unfortunately, nowadays sperm analysis and selection is done manually and the accuracy varies a lot depending on the level of expertise and experience, and this is a very time-consuming analysis process. This long process event got worsened due to COVID-19 precautions causing long patient waitlists worldwide.

The purpose of this study is to perform and evaluate the technical feasibility of SPERMAN® system that we are developing, therefore this study has a very important place for our technology to reach technical and operational competence as a commercial product.

Methods: In May and June 2021, a total of 39 human semen samples were sorted and analyzed with the SPERMAN® system according to sperm concentration and sperm motility parameters, and the results were compared with the swim up method followed by standard manual microscope method and computer-assisted sperm analysis system (CASA). Selection of healthiest sperm cells were performed on microfluidic chip of SPERMAN® system and standard swim up method.

Results: The coefficient factors between the analysis results with microscopy and CASA compared with SPERMAN® were 0.666 and 0.655 for sperm concentration and 0.662 and 0.658 for sperm motility, respectively. There were no issues with the clinical use of SPERMAN®. Device performance in classifying samples was positive ($< 15\times10^6$sperm/mL) and negative ($> 15\times10^6$ sperm/mL) for sperm concentration criteria, while positive (< 40%) and negative (> 40%) for sperm motility criteria. SPERMAN® sperm sorter system showed 92.6% sensitivity, 66.7% specificity and 84.6% overall accuracy.

Conclusion: This study shows SPERMAN® technology can easily obtain semen parameter information through automated manner and help infertile men's treatment therefore help increase efficiency of IVF procedures.

Poster 62

DEVELOPMENT OF A NOVEL CONTRACEPTIVE FOR MEN

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Presented By: Nadja Mannowetz, PhD

Introduction & Objective: Men are lacking contraceptive options that meet their needs and lifestyles. YourChoice Therapeutics is developing the small molecule YCT-529 into an oral, non-hormonal and reversible male contraceptive. YCT-529 acts as a retinoic acid receptor (RAR)-alpha antagonist thus inhibiting both spermatogenesis and spermiogenesis. YCT-529 has demonstrated a 99% contraceptive efficacy and full reversibility in mice at 10 mg/kg and sufficient and reversible sperm count reduction at 7.5 mg/kg. The next step is to assess the toxicity profile of YCT-529 to further develop it into a male contarceptive.

Methods: The initial safety profile of YCT-529 was assessed in vitro with target selectivity (cell-based luciferase assays), off-target screening (e.g., patch clamp and cAMP assays), and genotoxicity (Ames test) studies. Acute toxicity in animals was studied with single dose experiments in mice, rats, and dogs. Dose range finding (DRF) studies in rats and dogs were performed to evaluate sub chronic toxicity over a 14-Day dosing period.

Results: YCT-529 is (i) highly selective for RAR-alpha (the respective IC_{50} was 6.7 nM against RAR-alpha and > 3700 nM against RAR-beta and RAR-gamma; this was assessed with luciferase assays), (ii) not considered a hERG inhibitor (the IC_{50} was > 30 μM as assessed with patch clamp) and (iii) has no genotoxic potential (negative Ames test). Single oral dose studies showed that the respective maximum tolerated dose in mice, rats and dogs was > 1000 , 750 and > 500 mg/kg. Repeated oral dosing for 14 days demonstrated that rats and dogs tolerated 50-75 and 25 mg/kg, respectively. Using simple dose conversion between species, these values represent 13-20X and 22X multiples over mouse efficacy (7.5 mg/kg).

Conclusion: YCT-529 has demonstrated potent and reversible efficacy in mice and an initial safety profile with an over 10X safety margin in rats and dogs. With established efficacy and safety in vivo, it is the logical next step to rapidly accelerate the YCT-529 program towards first-in-human clinical trials. A pre-IND meeting with the FDA is scheduled for Q1 of 2022. IND-enabling studies - (i) 28-Day toxicity study in rats and dogs, (ii) rat respiratory study, (iii) dog cardiovasular study and (iv) rat functional observation battery - will commence in Q1/Q2 of 2022, and a Phase 1 clinical trial will begin in Q3 or Q4 of 2022.

Poster 63

NOVEL CONTRACEPTIVE METHOD: BLOCKING OF SERINE PROTEASE ACTIVITY TO PREVENT SEMEN LIQUEFACTION

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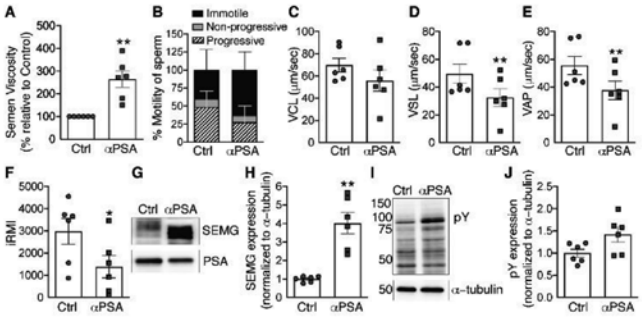
Presented By: Wipawee Winuthayanon, BSN, PhD

Introduction & Objective: Semen liquefaction is a biochemical process governed by prostate-specific antigen (PSA; a serine protease enzyme). PSA cleaves gel-forming proteins (semenogelins; SEMGs) and changes the semen from gel-like to watery viscosity. Highly viscous semen and abnormal liquefaction reduce sperm motility and contribute to infertility. However, specific inhibition of PSA activity has not been tested as a potential contraceptive method for blocking semen liquefaction. The objective of this study is to determine whether specific inhibition of PSA activity could be developed as a novel contraceptive target as there are no existing contraceptives that act by blocking semen liquefaction.

Methods: Methods: Human semen were collected from healthy subjects (n = 5-6 individuals/treatment). Fresh ejaculates were aliquoted and treated with PSA antibody for 30 minutes for viscosity assay and 4 hours for sperm motility assay. After incubation semen viscosity, sperm motility, and degradation of SEMGs were determined.

Results: We found that PSA antibody showed a dual contraceptive action where it 1) effectively inhibited liquefaction by preventing degradation of SEMGs resulting in a higher semen viscosity compared to control and 2) significantly decreased sperm motility by lowering the index of relative motility inhibition (iRMI).

Conclusion: In summary, inhibition of PSA activity using a neutralizing antibody can be developed for potential usage as novel, non-hormonal contraceptives for both men and women.



Anti-PSA inhibits semen liquefaction and sperm iRMI

Poster 64

IMPACT OF YOGA ON UNEXPLAINED INFERTILITY, COMORBID DEPRESSION AND QUALITY OF LIFE

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Presented By: Rajesh Kumar, MBBS, MD

Introduction & Objective: Unexplained infertility have been found to be associated with depression and decreased quality of life. Oxidative stress and decreased quality of DNA repair mechanisms are also involved in the pathogenesis of complex lifestyle and chronic diseases including depression and infertility. Yoga can decrease the oxidative stress and may impact the expression levels of genes involved in DNA repair. Evaluation of impact on yoga on oxidative stress, DNA repair genes, markers of neuroplasticity and quality of life in infertile men was done in this study.

Methods: Two group comparative preliminary longitudinal study with single blind trial had been done recruiting 75 infertile men not doing Yoga (Group A) and 75 infertile men doing Yoga (Group B). Semen and blood samples had been taken from the all the subjects in both the groups at days corresponding to 0 (baseline) and 45th day post yoga intervention. Seminal and Blood reactive oxygen species (ROS) and 8-OHdG levels had been used as oxidative stress markers. Expression levels of *OGG1*, *APE1*, *XRCC1* and *PARP1* genes in sperm and blood genome pre and post yoga and its validation via Real-Time PCR were calculated. We have also evaluated the quality of live by World Health Organization Quality-of-Life Scale and depression severity by Beck Depression Inventory-II scale. Levels of neuroplasticity markers like BDNF, DHEAS and sirtuin1 were also evaluated by ELISA kits.

Results: Significant decrease in ROS and 8OHdG levels was found in both semen and blood, significant Increased ($p < 0.0001$) expression levels of *PARP1* and *XRCC1*, decreased ($p < 0.0001$) expression of APE1 in sperm whereas significantly decreased ($p < 0.0001$) expression levels of *PARP1*, *XRCC1* and *APE1* were found in blood in yoga group. Significant reduction in depression severity (BDI-II score) and improved quality of life in all four domains by WHOQOL-BREF scale (physical, psychological, social, and environmental) was also found in Yoga group. Yoga also increased the levels of neuroplasticity markers like BDNF, DHEAS and sirtuin1 significantly ($p = < 0.005$).

Conclusion: Infertility is a complex psychosomatic disease that causes stress, anxiety and comorbid depression. Yoga not only decreases oxidative stress, DNA damage and positively modulates the epigenome but it also improves all four domains of quality of life as well reduces the severity of comorbid depression by positive influence on markers of neuroplasticity.

Poster 65

THE EFFECT OF LEPROSY ON MALE FERTILITY AND SEXUAL FUNCTION

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Presented By: Richard Mateo Mora, BS

Introduction & Objective: Leprosy, also known as Hansen's Disease, is a chronic infection with the bacterium *Mycobacterium leprae* or *Mycobacterium lepromatosis. Mycobacterium leprae* has been identified in the testicular tissue of men with leprosy. We sought to investigate the relationship between male infertility, sexual function, and leprosy.

Methods: Male patients at the LAC+USC Hansen's Clinic were surveyed regarding sexual and reproductive history and Sexual Health Inventory for Men (SHIM) scores. Primary outcomes were self-reported erectile function, sexual function, libido, number of children before and after leprosy diagnosis, paternity attempts, prior fertility work-up, and SHIM scores. SHIM scores were collected as a continuous variable and paired with non-leprosy age-matched controls. A two-sided Wilcoxon Signed Rank test was used to evaluate differences in SHIM scores.

Results: Seventeen men with leprosy were interviewed, and the mean age was 55 ± 25 years. The average age at the time of diagnosis was 43 ± 28. Nine (53%) men were sexually active. Six (35%) reported erectile dysfunction, seven (41%) reported ejaculatory dysfunction, and four (24%) reported impaired libido. Over half of men (53%, 9/17) reported de novo sexual dysfunction after their leprosy diagnosis. Four (24%) men reported experiencing primary infertility post leprosy diagnosis, and two (12%) had successfully conceived after their diagnosis. Only two (12%) men had prior semen analysis or hormone testing performed. SHIM scores were similar between leprosy and age matched controls, 18/25 and 22/25, respectively ($p = 0.255$).

Conclusion: Over half of men with leprosy experienced de novo sexual dysfunction post leprosy diagnosis, although it is impossible to know the reasons for this from our data. While over one-third of men

attempted paternity post leprosy diagnosis, only one-third of these had any type of fertility evaluation. Inquiring about sexual and reproductive history should be a part of the care of men with leprosy.

Poster 66

COVID-19 AND MALE REPRODUCTION: A SCIENTOMETRIC STUDY

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Presented By: Manesh Kumar Panner Selvam, PhD

Introduction & Objective: Coronavirus disease (COVID-19) pandemic adversely impacts the global human health. Over the past two years, there has been a tremendous surge in COVID-19 related scientific publications (both animal and human studies). Bibliometric and scientometric approaches have been used to understand the publication trend of COVID-19 related articles in different areas of biological sciences. However, such type of analysis on scientific literature still lacks in the area of male reproduction. The current scientometric study identifies the COVID-19 related research and further analyzes publication characteristics of articles published in the field of male reproduction and COVID-19.

Methods: The Scopus database was used to retrieve scientometric data (the number of publications, journals, countries, type of documents and subject area) related to human reproduction and COVID-19. The search was carried out on 11 January 2022 using keyword string: "COVID" OR "SARS-CoV-2″ OR "COVID-19″ AND "Reproducti*". Title, abstract and keywords were screened to identify the relevant articles. Animal studies, non-English articles and publications not related to the reproduction and COVID-19 were excluded from the analysis. Subsequent scientometric analysis was carried out on the publications related only to male reproduction and COVID-19 research.

Results: Literature search revealed a total of 328 articles related to COVID-19 and human reproduction. Majority of the articles were published from the USA (n = 89) followed by the UK (n = 44) and China (n = 43). Such research was mainly carried out in the field of medicine (66.1%, n = 297), biochemistry, genetics and molecular biology (18.3%, n = 82), and immunology and microbiology (4.2%, n = 19). Scientometric analysis indicated that 26.5% (n = 87) and 12.8% (n = 42) of the COVID-19 research was focused on male reproduction and assisted reproduction, respectively. Furthermore, only 25.3% (n = 22) contributed towards original research, whereas 74.7% (n = 65) were reviews and editorials. In-depth analysis revealed that 14 studies have investigated the presence of SARS-CoV-2 in semen.

Conclusion: There were only a small number of original research articles on male reproduction related to COVID-19. Majority of these articles evaluated semen parameters to assess semen quality in men infected with SARS-CoV-2. A substantial increase in the research is required to decipher the mechanism(s) underlying SARS-CoV-2 infection associated impairment of semen parameters which in turn could affect the male reproductive health

Poster 68

LIVE SPERMATOZOA MORPHOMETRY BY DIGITAL HOLOGRAPHIC MICROSCOPE

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Presented By: Maciej Kurpisz

Introduction & Objective: Digital holographic microscopy (DHM) allows to obtain quantitative information about the topographic profile of the live object and offers non-destructive, noninvasive, quantitative, label-free, three-dimensional measurements. This method let us study the sperm population directly from the sample droplet and not only from the focal plane of the microscope as in classical optical microscopy. The newly implemented three-dimensional sperm morphological parameters (*head height*, *acrosome/nucleus height*, *head/midpiece height)* were included in morphological assessment of semen samples from fertile and infertile individuals.

The aim was to investigate the status of sperm morphology in fertile and infertile men by means the holographic method using live spermatozoa after liquefaction or selection.

Methods: The morphology of human semen from 10 fertile and 12 infertile men was studied using a digital holographic microscope DHM-T1000 (Lyncée Tec SA, Lausanne, Switzerland) and the software Koala V4. New parameters: *head height*, *acrosome/nucleus height* and *midpiece/nucleus height* were involved to morphological assessment. For the first time, DHM was applied to investigate morphology after swim-up selection (30 and 60 minutes incubation) and gradient centrifugation technique.

Results: The morphological profiles of spermatozoa obtained by DHM did not differ in statistically significant way between the individuals when we compared the classical (*head length*, *head width*, *midpiece length*, *tail length*) parameters. However, the wider statistical range of invented DHM values (*head height*, *acrosome/nucleus height*) was observed in infertile men.

Statistical comparison showed differences between samples depending on the method of sperm separation (swim-up 30 minutes; swim-up 60 minutes, Percoll 90%). We compared the above separation methods with native sperm suspension. Any single WHO or DHM parameter studied was not able to distinguish spermatozoa before and after selection. During selection, the most statistically significant differences were observed after separation with a 90% Percoll gradient and a 60-minute “swim-up” procedure versus ‘native’ unfractionated samples. This shows that the developed methodology can be efficiently used for the selection of morphologically sound spermatozoa.

Conclusion: 3D parameters provide new information on live sperm morphology that may be helpful in assessing fertility and that might differentiate their fertilization ability with respect to external factors applied.

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Poster 70

ANTI-CANCER EFFECT OF WITHANIA SOMNIFERA ON PROSTATE CANCER CELLS IS MEDIATED BY REACTIVE OXYGEN SPECIES LEVELS

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Presented By: Pradeep Chaturvedi, PhD

Introduction & Objective: Prostate cancer is the fifth leading cause of cancer related deaths worldwide. To lessen the burden of side effects of conventional treatments, ayurvedic practitioners are regularly investigating medicinal plants for their potential anti-cancer effect. Withania Somnifera is an Ayurvedic plant with proven anti-cancer effect in several cancers. The current study investigated the effect of Withania Somnifera (WS) extract on prostate cancer cells in vitro.

Methods: Androgen independent prostate cancer cell line (PC3), androgen dependent prostate cancer cell line (22Rv1) and normal prostate gland epithelial cell line (RWPE-1) were used in this study. The cells were treated with different doses of WS root extract ranging from 25 to 400 μg/mL for 24 , 48 or 72 hours and cell viability was measured by MTT assay. The effect of WS extract on levels of reactive oxygen species (ROS) was measured by DCFDA assay. Levels of LC3B-I/LC3B-II proteins, microtubule associated proteins and molecular markers of cells undergoing autophagy, were measured in PC3 cells by Western blot.

Results: WS extract significantly inhibited cell viability of PC3 and 22Rv1 cell lines in a dose-dependent manner. The IC_{50} value of WS extract at 48 hours for PC3 cells was much lower than that of 22Rv1 and RWPE-1 cells. This suggests that androgen independent PC3 cells are more vulnerable to WS extract. Previous studies have shown that higher levels of ROS promote cancer progression by inducing proliferation and migration of cancer cells, therefore effect of WS extract on ROS levels was measured. PC3 cells treated with IC_{50} concentration of WS extract had significantly decreased levels of ROS when compared to untreated cells but no significant decrease when compared to vehicle-treated cells. However, 22Rv1 cells treated with IC_{50} concentration of WS extract showed significant decrease in levels of ROS when compared to either untreated or vehicle treated cells. Ratio of LC3B-II and LC3B-I protein in PC3 cells treated with WS extract indicated that autophagy was inhibited by WS extract.

Conclusion: Treatment with WS extract significantly decreased cell viability of both PC3 and 22Rv1 cells, possibly by regulating intracellular ROS levels. However, the cytotoxic effect of WS extract was not mediated via autophagy but possibly involves some other mechanism like apoptosis etc.

Poster 71

IMPACT OF THE COVID-19 PANDEMIC ON INITIAL MALE INFERTILITY EVALUATION AND VASECTOMY CONSULTATIONS

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Presented By: Moshe Wald, MD

Introduction & Objective: The first U.S. laboratory-confirmed case of COVID-19 in the U.S. was confirmed by the Centers for Disease Control and Prevention on January 20, 2020. COVID-19 was declared a global pandemic by the World Health Organization on March 11, 2020. Starting that month, this pandemic had a remarkable effect on hospitals' function across the U.S, and on the ability and willingness of patients to obtain elective health services. Our study assesses the impact of the COVID-19 pandemic on the number of men who arrived for initial infertility evaluation or vasectomy consultation during the first year of this pandemic.

Methods: The EPIC program SlicerDicer was used to identify all men seen at the University of Iowa's urology clinic for initial infertility evaluation or vasectomy consultation between March 1, 2016, and February 28, 2021. Patient cohort was divided to those seen yearly (March 1 through the end of February on the following year) during the study period. Number and age of patients seen yearly for these indications before and after March 1, 2020 (the presumed date after which COVID-19 started to have an impact on healthcare in the United States), were assessed.

Results: From March 2016 through February 2020, the average yearly numbers of patients who presented for new infertility evaluations and for vasectomy consultations were 60 (49 – 73) and 76 (49 – 116), respectively (Table 1).

Table 1:

	New Infertility Evaluations	New Vasectomy Consults	Age (average)
March 2016 through February 2017	50	49	24-58 (40.22)
March 2017 through February 2018	73	71	25-64 (39.05)
March 2018 through February 2019	49	68	25-69 (40.2)
March 2019 through February 2020	67	116	18-58 (38.6)
March 2020 through February 2021	74	78	23-63 (37)

Table 1

From March 1, 2020, through the end of February 2021, 74 men were seen for new infertility evaluations, while 78 men presented for vasectomy consultations. Patient's age in this group ranged from 23 to 63 years (average 37 years).

Wilcoxon rank sum test was used for age difference between event type (infertility or vasectomy). Age for vasectomy patients was significantly higher than infertility in 2016 and 2017. Age for vasectomy

patients was lower than that of infertility patients in 2018, 2019, and 2020, but these were not significant differences.

Conclusion: In this study the number of men who presented for infertility evaluation and for vasectomy consultations during the first year of the COVID-19 pandemic was comparable to the number of patients who presented for those indications during the preceding four years. These findings may suggest prompt institutional adjustment for the accommodation of elective clinic visits during the first year of this pandemic.

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FUNCTIONAL ANALYSIS OF THE HEDGEHOG PATHWAY IN THE MAMMALIAN SPERM

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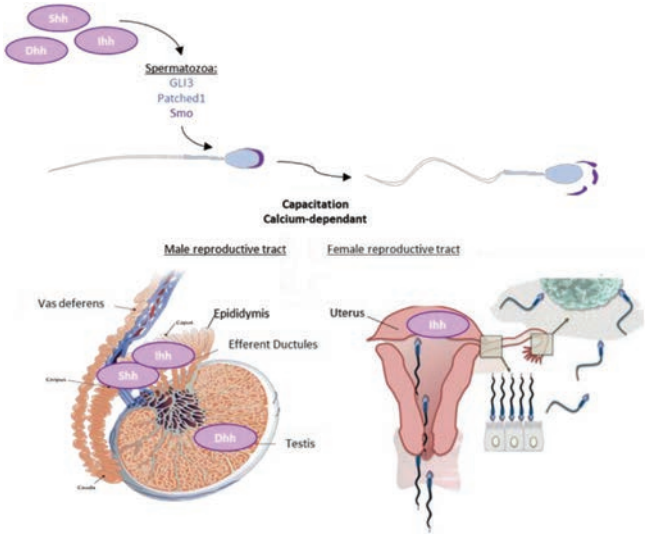
Presented By: Laura Girardet

Introduction & Objective: The Hedgehog (Hh) signaling pathway is essential for the homeostatic control of organs. While previously thought to be exclusively transduced through non-motile primary cilia, a recent study indicated that Hh signaling could modulate cAMP concentration and ciliary beating in the airway. Since systemic blockade of Hh signaling indirectly reduces sperm motility in vivo, we hypothesized that Hh agonists could directly modulate sperm functions in vitro through a non-canonical pathway.

Methods: The presence and localization of Hh factors, including the receptors Patched1 and Smoothened, and Gli3 were investigated by immunofluorescence on murine, bovine, and human spermatozoa. To understand if this pathway was important for sperm functions, capacitation, motility (CASA measurement), acrosome reaction (PNA-FITC staining), viability (propium iodide staining), intracellularly calcium (INDO-1, FACS) and cAMP were assessed in spermatozoa incubated with Hh activator (SAG, 500 nM) or inhibitor (Cyclo, 50 μM).

Results: Hh signaling depends on the secretion of the ligands (Dhh, Shh or Ihh) by cells that bind to Patched1 at the cilia membrane, allowing Smoothened and Gli3 activation. Both Shh and Ihh were detected in the epididymal fluid and Ihh has been demonstrated in the female uterus. In this study, Patched1, Gli3 and Smoothened were demonstrated in spermatozoa from the three species, mostly within the intermediate piece of the flagellum and the sperm head. Interestingly, Smoothened was found in the sperm acrosome in the three species, suggesting a role in its regulation. In bulls, activation of the Hh pathway promotes the acrosome reaction (22% control vs. 39% with SAG, $n = 5$, $p < 0.01$). In humans, activation of the Hh pathway greatly reduces sperm hyperactivity (16% control vs. 7% with SAG, $n = 4$, $p < 0.01$) while its inhibition decreases acrosomal reaction (control 32% vs. 23% with Cyclo, $n = 7$, $p < 0.001$). Moreover, although these changes were not accompanied by a change of intracellular cAMP, an increase in intracellular calcium was induced by the activation of the Hh pathway in human spermatozoa.

Conclusion: Our results suggest that the Hh pathway regulate sperm acrosome reaction and motility independently from cAMP. Although further studies are needed to determine if Hh activation/inhibition could impact in vitro fertilization success, this study provides new avenues on the control of male reproductive functions.



Hedgehog signalling in mammalian spermatozoa

Poster 73

CRYOPRESERVATION MODIFIES THE PHOSPHOPROTEOME OF THE STALLION SPERMATOZOA

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Presented By: FERNANDO PEÑA, PhD

Introduction & Objective: Spermatozoa are terminal highly differentiated cells with a very little capacity for transcription of new proteins. Due to these characteristics, spermatozoa are highly dependent of the incorporation of new proteins through micro-vesicle trafficking and post translational modifications of existing proteins. Protein phosphorylation is a post translational modification (PTM), involved in the regulation of most biological processes. In the spermatozoa plays a major role in many important functions such acquisition of motility upon ejaculation, capacitation, and fertilization

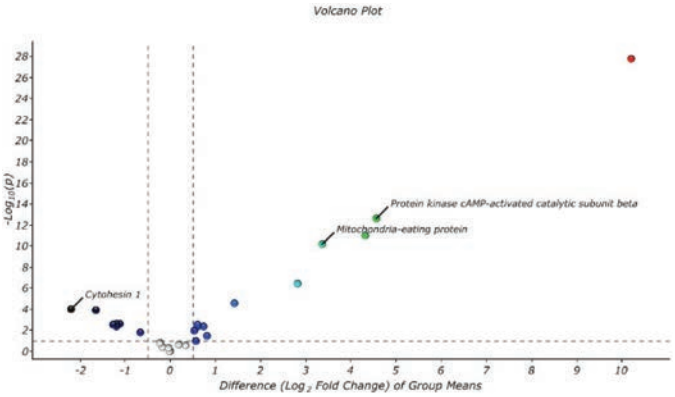
Methods: Both fresh and frozen thawed (FT) samples of spermatozoa were washed three times using PBS (600 g x 10′). After this samples were subsequently pelleted and kept frozen at -80°C until further analysis. Phase contrast microscopy was used to assure purity of the samples. A UHPLC/MS system consisting of an Agilent 1290 Infinity II Series UHPLC (Agilent Technologies, Santa Clara, CA, USA) fitted with an automated multisampler module and a high-speed binary pump, coupled to an Agilent 6550 Q-TOF Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA) using an Agilent Jet Stream Dual

electrospray (AJS-Dual ESI) interface was used to separate and analyze the samples

Results: A total of 363 phosphoproteins were identified Enrichment analysis of this set of proteins revealed the gene ontology (GO) terms nucleotide binding (GO 0000166; $p = 4.109x10^{-2}$), nucleoside phosphate binding (GO 1901265; $p = 4.109x10^{-2}$) and sperm fibrous sheath (GO 0035686; $p = 1.522x10^{-3}$) . *Cryopreservation reduces the amount of specific phosphoproteins.* We investigated the effect of freezing and thawing on the stallion sperm phosphoproteome; cryopreservation significantly reduced the presence of the phosphoproteins; *Ca2+binding tyrosine phosphorylation regulated, protein kinase cAMP-activated catalytic subunit beta, mitochondria eating protein. AKAP4, AKAP3, family with sequence similarity 71 member B, mitochondrial zinc maintenance protein and glyceraldehyde 3 phosphate dehydrogenase.*

Cryopreservation increases the amount of specific phosphoproteins. On the contrary specific phosphoproteins were more abundant in frozen thawed samples, including the *ABC transporter ATP-binding protein permease, RAS P21 protein activator 2, Cytohesin 1, MAP3K19, PNP_UDP_1 containing domain, coiled coil and C2 domain containing 2A and DUF 907 domain containing protein.*

Conclusion: In this study, changes in the phosphoproteome provide a molecular explanation to the reduced functionality of the spermatozoa after thawing.



Volcano plot showing phosphoproteins in stallion spermatozoa

Poster 74

METABOLIC CHANGES DURING MOUSE AND HUMAN SPERMATOZOA CAPACITATION

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Presented By: Melanie Balbach, PhD

Introduction & Objective: Mammalian sperm are stored in the epididymis in a dormant state. Upon ejaculation, they must immediately start producing sufficient energy to maintain motility and support capacitation. While this increased energy demand during capacitation is well-established, it remains unclear how mammalian sperm modify their metabolism to meet this need.

Methods: Using an extracellular flux analyzer, metabolomics and metabolic flux analysis we compare the metabolism of non-capacitated and capacitated mouse and human spermatozoa, sperm treated with soluble adenylate cyclase (sAC) and protein kinase A (PKA) inhibitors and sperm from sAC knockout mice.

Results: By combining these techniques we show that glycolysis and oxidative phosphorylation increase during capacitation in mouse and human spermatozoa and that there is a functional link between glycolysis and oxidative phosphorylation. We discovered that the flux through glycolysis, pentose phosphate pathway and citrate cycle is altered during capacitation and that also endogenous substrates like fatty acids and amino acids are utilized for energy production. Furthermore, we identified a subset of glycolytic steps regulated by sAC.

Conclusion: Our study provides new insights into the energy production during mammalian sperm capacitation and experimental evidence for a link between sAC/PKA-regulated signaling pathways and mammalian sperm metabolism.

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"SUPPLEMENTATION WITH AçAI BERRY (EUTERPE OLERACEA MARTIUS) REVERSES THE DAMAGE IN SPERM DNA, ACROSOME AND MITOCHONDRIA CAUSED BY SENESCENCE IN A D-GALACTOSE RAT MODEL"

Tereza Simões-Ferreira[1], Yanka DA SILVA[1,2], Samuel Fortini[1,3], Thalita de Rosa[1,4], Marina Araújo[1,4], Sandra Miraglia[1], Ana Clara Gomes[1], Vanessa Vendramini[1]

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Presented By: Vanessa Vendramini, PhD

Introduction & Objective: Age-related metabolic diseases are common causes of reduced fertility, a pattern intimately linked with lower quality of the sperm DNA content. D-galactose administration in rodents has been used as a model for mimicking systemic increase in inflammatory and pro-oxidative status typically seen in senescence, with a known impact in sperm motility, morphology and genes related to nuclear integration. Nutraceuticals containing anthocyanins, such as açai berry (*Euterpe oleracea* Martius), are known as effective adjuvants in controlling or preventing diseases related to metabolic syndrome, mainly through antioxidant activity. Thus, our study aimed to find out if lyophilized açaí could be a therapeutic strategy in reversing alterations in sperm DNA and functionality caused by the acceleration of reproductive decay in rats.

Methods: Senescence acceleration was induced in 70-day-old male Wistar rats using daily doses of 200 mg/kg b.w. of D-galactose for 8 weeks by gavage, and a group of the senescent rats was

supplemented with 200 mg/kg of lyophilized açai berry for a half of this period. The animals were divided into senescent (DG, n = 6), supplemented with açai (DGA, n = 6), and sham control (C, n = 4) groups. The animals were tested for blood glucose level and weighed periodically. Reproductive organs were collected at the end of treatment, weighed, and sperm from the epididymis cauda was sampled and stored for DNA fragmentation test (SCSA), protamination (CMA3), lipid peroxidation (BODIPY C11), acrosome integrity (PNA) and mitochondrial mass (MitoTracker Green), all performed using flow cytometry.

Results: Rats from DG group showed opaque, dry and thin fur, characteristics not seen in the DGA and C groups. In the DG group there was a statistically significant increase in the weight of the epididymis, and increased numbers of spermatozoa with DNA fragmentation and altered acrosome, accompanied by higher levels of lipid peroxidation and reduced mitochondrial mass. Conversely, rats supplemented with lyophilized açai had improved integrity of the chromatin, acrosome, and mitochondrial mass when compared to the DG group.

Conclusion: The results presented here suggest a potential role of the supplementation with lyophilized açai to reverse the damages caused by senescence induced by D-galactose on sperm quality and functionality. However, it seems that the increase in mitochondrial activity caused by açai may have contributed to an increased production of pro-oxidative molecules.

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LACK OF EFFECT ON MITOCHONDRIAL FUNCTION AND LIPID PEROXIDATION IN RAT SPERM EXPOSED TO METHYLPHENIDATE HYDROCHLORIDE DURING ADOLESCENCE

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Presented By: Ana Clara Gomes, MS

Introduction & Objective: Methylphenidate hydrochloride (MPH) is a psychostimulant drug that acts by inhibiting the reuptake of dopamine and noradrenaline. It is commonly prescribed for the treatment of Attention Deficit Hyperactivity Disorder (ADHD), but it has been widely used as a performance enhancer without medical indication. Up to the moment, the available studies show that the effect of MPH in human spermatozoa is restrict to routine sperm parameters with no evidence in reducing male fertility, while in other species some reports suggest alterations in spermiogenesis, motility, Leydig cell function and also in the fertility. Besides that, behavioural effects of paternal-mediated MPH exposure was detected on the offspring and great-grandoffspring of fish. The presence of dopamine and noradrenaline receptors in testicular tissue suggests that MPH may have a direct action on this tissue. In a previous study we observed an increase in sperm DNA fragmentation, a reduction in fertility and in the quality of embryos conceived by animals treated with this psychostimulant during adolescence.

Methods: Thus, the present study aimed to verify if there is an effect of MPH on the mitochondrial sheath and spermatic membranes after treatment during adolescence in rats. Six Wistar male rats at 38 days of age were treated either with 5 mg/kg body weight of methylphenidate hydrochloride, in a single daily dose for 30 days, via gavage (treated group, n = 3); or with distilled water-only, using the same protocol (control group: n = 3). At 70 days of age, sperm was collected from the epididymis cauda for lipid peroxidation (BODIPY C11) and mitochondrial mass (MitoTracker Green), all performed using fresh samples immediately analyzed in a flow cytometer FACS Canto II.

Results: MPH did not affect the mitochondrial function in the spermatozoa of treated animals, neither generate lipid peroxidation in sperm membranes.

Conclusion: Thus, we conclude that the decrease in sperm quality may not be a consequence of mitochondrial damage or via oxidative stress at the testicular level, what corroborates other previous findings related to MPH-induced increase in sperm motility in vivo and in vitro. This reinforces our hypothesis that MPH may be acting through other mechanisms, for instance the inhibition of epigenetic molecules that act in the spermatic DNA compaction process.

Poster 77

COUNTING HOECHST-STAINED SPERM HEADS IN ALDEHYDE-FIXED SEMEN

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Bedford Research Foundation, Bedford, MA, USA

Presented By: Alexander Hauser, BS

Introduction & Objective: To improve patient compliance with post-vasectomy semen analyses, an aldehyde-based fixative developed two decades ago to maintain cells in semen in suspension for quantitation has been adapted to a home collection kit ("PVSA"). The threshold of sperm detection is 15,000 sperm/mL of undiluted semen. Because a high background of fixed protein, somatic cells, and sub-cellular particles can obscure sperm detection in some specimens, Hoechst staining of sperm head DNA has been compared to standard hemacytometer sperm counts.

Methods: Semen specimens collected in a condom by men at their convenience are added to a small bottle of fixative and mailed to the laboratory for sperm assessment. Standard hemacytometer sperm counts that take into account the dilution by fixative have been compared to an inspection of fluorescent photo-scans generated by an EVOS M-7000 scanning microscope. In addition, counts obtained by software (Celleste) - entrained to count sperm heads and somatic cell nuclei, were compared with counts obtained by standard hemacytometer evaluation. Fixed cells were immunostained to identify the class of somatic cells.

Results: Visual evaluation of 177 fluorescent photo-scans agreed with hemacytometer counts of specimens with low background. It

detected more sperm heads with fluorescent DNA in samples with a high fixed particle background. Entrained software (Celeste) could distinguish between sperm heads and somatic cell nuclei and quantify each. However, with some cross-over between the lobed nuclei of granulocytes and sperm heads in the 5% of post-vasectomy specimens contain cells. Immunostaining the post-vasectomy specimens containing cells distinguishes the class of leukocytes from other somatic cells.

Conclusion: Fluorescent-staining sperm head DNA in aldehyde-fixed, mail-in semen specimens improves the accuracy of sperm counts in post-vasectomy specimens with a high background. In addition, the identity of fixed cells in the specimens may reveal valuable information about male reproductive health after vasectomy.

Poster 78

MODIFYING CAPACITATION ABILITY THROUGH LIFESTYLE CHANGES

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Presented By: Fady Sharara, MD

Introduction & Objective: Reports suggest that traditional semen analysis parameters have been declining, although impacts on male fertility are unclear. Lifestyle practices and environmental exposures might be contributing to such declines. Traditional semen analysis (SA) assesses spermatogenesis, accessory gland contributions, and descriptive parameters of motility, concentration, and morphology. However, it fails to evaluate sperm function and to diagnose at least half the cases of male infertility. To fertilize, sperm must complete the process of capacitation. Cap-Score™ not only quantifies capacitation ability and functionally assess male fertility, it also prospectively predicts a man's ability to generate pregnancy.

Objective: Determine the ability of lifestyle changes to moderate capacitation ability and SA measures.

Methods: Cap-Score was determined in blinded fashion for 38 men seeking fertility assistance. Their lifestyle was subsequently changed by quitting use of tobacco, marijuana, or alcohol; avoiding laptops on laps or Jacuzzis/saunas; losing weight if obese; increasing Vitamin D intake (at least 2k/day); and starting supplements (Androferti (n = 30; vitamins C, E, B12, Folate, Zinc, selenium L-carnitine, and coenzyme Q10), or Conception XR (n = 8; vitamins C, E, D, Folate, Zinc, selenium, and Lycopene)). A second blinded analysis was done approximately 10 weeks after starting this change. To determine supplement impact, two-sample t-tests were done on the difference between the first and second reading. The impacts of lifestyle changes were determined using paired t-tests comparing the first to the second analysis. Linear regression was used to assess the relationship between Cap-Score and strict normal morphology.

Results: Both supplements had a similar impact on all measures ($p > 0.05$). An increase in Cap-Score from 24.2±1.2 to 27.9±1.2 ($p = 0.016$), corresponding to a 20% increase in a man's probability of generating a pregnancy, was observed after lifestyle changes. Lifestyle changes had no impact on the following SA measures: semen volume ($p = 0.527$), sperm concentration ($p = 0.547$), sperm motility ($p = 0.202$), and total motile sperm ($p = 0.535$). In contrast, strict normal morphology improved (1.7±0.2 to 3.3±0.5; $p = 0.001$). No relationship was detected between Cap-Score and strict normal morphology before ($p = 0.566$) or after ($p = 0.156$) lifestyle changes.

Conclusion: These data support the view that promoting a man's overall health by quitting smoking, drinking, marijuana and losing weight, in combination with nutritional supplements, is linked to changes in sperm capacitation ability and an increase in male fertility.

Poster 79

PROTEOMIC ANALYSIS OF TESTICULAR AND EPIDIDYMAL SPERM FROM BULLS WITH A HIGH PERCENTAGE OF MIDPIECE ABNORMALITIES

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Presented By: Arabela Viana, DVM, MS

Introduction & Objective: Morphological abnormalities in ejaculated sperm indicate alterations in testicular and epididymal functions. Midpiece defects including aplastic midpiece defect (AMD) may compromise male fertility by reducing sperm motility and generating oxidative stress. Herein, we aimed to characterize the protein profile of bovine sperm with AMD, from their production in the testis to their maturation in the epididymis.

Methods: Testis and caput and cauda epididymis from six bulls (*Bos indicus*) were included in this study. The animals were divided into two groups, classified as satisfactory potential breeders (control; n = 3) and unsatisfactory potential breeders (AMD; n = 3), due to the presence of AMD in the semen. The organ fragments were placed in Petri dishes containing BWW media. After incubation, spermatozoa were recovered, filtered, and centrifuged in Percoll® 30%. The cells were washed in red blood cell lysis buffer and BWW medium until removing contaminating cells. Spermatozoa from control and AMD bulls were pooled, and their proteins were extracted and analyzed by LTQ Orbitrap XL mass spectrometry. Data were processed (Peaks Studio 8.5) and proteins were identified using Uniprot and NCBI repositors. A log 2-fold change (log2FC) was carried out to identify differentially expressed sperm proteins between control and AMD bulls. The enrichment of metabolic pathways was accessed using the DAVID platform.

Results: From 379 identified proteins, 199 were found in both sperm phenotypes, 33 were specifically found in normal sperm, and 147 in AMD sperm. Thirty-one proteins from the total of 199 were upregulated in AMD sperm, whereas 17 were down-regulated (Table 1). Interestingly, 13 proteins were highly upregulated in sperm from the caput epididymis, exhibiting log2FC greater than 30. Moreover, sperm acrosome membrane-associated protein 3, involved in sperm-egg fusion, was downregulated in caput epididymal sperm and upregulated in cauda epididymal sperm. Functional clustering analysis showed a decrease in oxidative phosphorylation pathway in control sperm. Pathways related to protein degradation and tissue remodeling, as well as synaptic vesicle cycling and collecting duct acid secretion, were enriched in AMD sperm.

Conclusion: Our preliminary data showed relevant differences among proteins expressed in AMD sperm of bulls, with a decrease in oxidative phosphorylation pathway. Differences in protein expression between sperm from caput and cauda regions suggest that AMD may be related to epididymal dysfunction.

Table 1. List of up and down-regulated proteins of testicular and epididymal (caput and cauda regions) sperm from bulls with aplastic midpiece defect (AMD).

Accesion	Protein description	Regulation	Log2FC
Testicular sperm			
P52193	Calreticulin	Up	9.51
P00442	Superoxide dismutase [Cu-Zn]	Up	8.46
Q3SZC0	Enhancer of rudimentary homolog	Up	7.86
Q32LE3	Centrin-1	Up	7.49
P56965	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	Up	6.61
P02721	ATP synthase-coupling factor 6, mitochondrial	Up	6.16
P52505	Acyl carrier protein, mitochondrial	Up	5.97
P28801	Glutathione S-transferase P	Up	5.94
Q3MHM5	Tubulin beta-4B chain	Up	5.89
P68401	Platelet-activating factor acetylhydrolase IB subunit alpha2	Up	5.88
P12624	Myristoylated alanine-rich C-kinase substrate	Up	5.85
P00171	Cytochrome b5	Up	5.46
Q5E9G3	Proteasome activator complex subunit 2	Up	5.30
P17697	Clusterin	Up	5.07
Q3SZE2	Prefoldin subunit 1	Down	-5.21
P55206	C-type natriuretic peptide	Down	-6.44
Epididymal sperm (caput region)			
Q95114	Lactadherin	Up	32.17
Q2T9X5	Uncharacterized protein C7orf61 homolog	Up	31.78
Q9XSJ4	Alpha-enolase	Up	31.77
P29392	Spermadhesin-1	Up	31.61
Q2TA43	Actin-related protein T2	Up	31.47
P61283	Barrier-to-autointegration factor	Up	31.33
Q2TBQ6	Heat shock protein beta-9	Up	31.12
Q2T9U2	Outer dense fiber protein 2	Up	30.61
O46415	Ferritin light chain	Up	30.58
Q2YDG7	Sperm acrosome membrane-associated protein 1	Up	30.49
P61282	NEDD8	Up	30.29
P00442	Superoxide dismutase [Cu-Zn]	Up	30.26
A0JNM6	Lysozyme-like protein 1	Up	30.21
P63258	Actin, cytoplasmic 2	Up	28.69
Q3MHW9	NADH-cytochrome b5 reductase 1	Down	-5.11
P00428	Cytochrome c oxidase subunit 5B, mitochondrial	Down	-5.21
Q08DD1	Arylsulfatase A	Down	-5.45
P00126	Cytochrome b-c1 complex subunit 6, mitochondrial	Down	-5.49
A6QQ77	Sperm acrosome membrane-associated protein 3	Down	-5.63
P00426	Cytochrome c oxidase subunit 5A, mitochondrial	Down	-5.83
P00829	ATP synthase subunit beta, mitochondrial	Down	-6.12
Q32L79	Insulin-like peptide INSL6	Down	-6.26
Q2T9N7	Lysozyme-like protein 4	Down	-6.45
O77780	Disintegrin and metalloproteinase domain-containing protein 2	Down	-6.71
Q2KJE5	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	Down	-7.23
Q3SYS7	IQ domain-containing protein F1	Down	-7.41
Q2YDI9	Ferritin, mitochondrial	Down	-7.56
Epididymal sperm (cauda region)			
Q2HJ86	Tubulin alpha-1D chain	Up	9.61
A6QQ77	Sperm acrosome membrane-associated protein 3	Up	6.39
Q2TA14	Lysosomal Pro-X carboxypeptidase	Up	6.00
P82292	Spermadhesin Z13	Up	5.90
P62157	Calmodulin	Up	5.63
P19858	L-lactate dehydrogenase A chain	Down	-5.74

Up and down-regulated proteins of bull sperm with AMD.

Poster 80

OVIDUCT 3′-O-SULFATED LEWIS X TRISACCHARIDE DECREASES THE ABUNDANCE OF AN ELECTRON CARRIER IN THE ELECTRON TRANSPORT CHAIN TO REDUCE ROS ACCUMULATION AND INCREASE PORCINE SPERM LIFESPAN

Jennifer Hughes, David Miller
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Presented By: Jennifer Hughes, PhD

Introduction & Objective: Sperm storage in the female tract is a conserved reproductive strategy across numerous species and phyla. In many mammals, sperm are retained in the lower oviduct to form a reservoir that provides sperm to the site of fertilization. Although the reservoir extends sperm lifespan, it is unclear how this is accomplished. To be retained in the oviduct, porcine sperm bind to N-linked glycans on the oviduct epithelium containing either the Lewis X trisaccharide or a 6-sialylated biantennary motif. Like binding to oviduct epithelial cells, binding to oviduct glycans attached to beads lengthens sperm lifespan compared to free-swimming sperm. The mechanisms by which sperm lifespan is prolonged are unclear. We used metabolomic analysis and flow cytometry to assess the effects of binding 3′-O-sulfated Lewis X ($suLe^X$), which has a higher affinity for sperm than non-sulfated Le^X, on sperm function during a 4 hr capacitation time.

Methods: Spermatozoa were washed and resuspended in dmTALP medium containing soluble 0.1 ug/mL $suLe^X$, 0.1 ug/mL 3′-O-sulfated Lewis A trisaccharide (an isomer of Lewis X), both attached to a 20 kDa polyacrylamide chain, or vehicle control. Sperm were then incubated in capacitating conditions for 0.5, 1, 3, or 4 hours. Targeted metabolomic analysis was performed and data were analyzed with MetaboAnalyst. Flow cytometry was performed to measure viability using propidium iodide and reactive oxygen species (ROS) using CellROX.

Results: There was a transient reduction in 4-hydroxybenzoic acid in $suLe^X$-treated sperm at 0.5 hour incubation. After incubation for 4 hours, CellROX staining was also reduced in sperm treated with $suLe^X$. Similarly, $suLe^A$ also reduced the amount of ROS at 4 hours. 4-hydroxybenzoic acid remained lower in $suLe^X$ treated sperm at later times, but the magnitude of change from control was reduced compared with 0.5 hour.

Conclusion: The reduction in 4-hydroxybenzoic acid, a precursor of ubiquinone, an electron carrier in the electron transport chain, suggests that $suLe^X$ binding reduces the activity of the electron transport chain and perhaps mitochondrial function. This occurs at 0.5 hours of incubation with $suLe^X$. In concordance with this, at the 4 hr time, $suLe^X$-treated sperm had lower ROS. We propose that oviduct glycans reduce electron transport early in capacitation, leading to less electron leakage and less ROS production later in capacitation, lengthening sperm lifespan.

Poster 81

ROLE OF TSPO IN MITOCHONDRIAL FUSION AND FUNCTION IN MA-10 MOUSE TUMOR LEYDIG CELLS

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Presented By: Samuel Garza, BS, MS

Introduction & Objective: Testicular Leydig cells are the main site of testosterone production in men and contribute to the maintenance of circulating testosterone levels. Of the proteins involved in testosterone biosynthesis, the outer mitochondrial membrane translocator protein (TSPO 18 kDa) possesses a cholesterol recognition amino acid consensus (CRAC) binding domain which is thought to be critical for cholesterol translocation into mitochondria for testosterone biosynthesis. Although evidence establishes a connection between TSPO and steroid biosynthesis, the mechanisms which translocate cholesterol to the inner mitochondrial membrane where CYP11A1 is located are not well understood. Removing TSPO diminishes steroid biosynthesis and disrupts mitochondrial membrane morphology, suggesting that TSPO regulates mitochondrial fusion dynamics. This indicates that mitochondrial fusion may be involved in steroid biosynthesis.

Methods: The MA-10 mouse tumor Leydig cell line and the TSPO-depleted MA-10 sub-cell line, nG1 (*Fan et al., Endocrinology, 2018;159:1-17*), were used to interrogate the role of TSPO in mitochondrial function. To enhance mitochondrial networks, cells were treated with the cell-permeable mitochondrial fusion promoter 4-Chloro-2-(1-(2-(2,4,6-trichlorophenyl) hydrazono) ethyl) phenol (M1). To confirm the results obtained with M1, we overexpressed the inner mitochondrial membrane GTPase optic atrophy 1 (*Opa1)* gene, which encodes the mitochondrial membrane fusion enzyme, OPA1. Steroid production, mitochondrial function and morphology were assessed by ELISA, Seahorse Xfe96 analysis and transmission electron microscopy (TEM), respectively.

Results: Data revealed a quiescent energy profile in TSPO-depleted cells. nG1 cells had reduced cellular respiration, oxygen consumption rate, ATP production and mitochondrial proton leak, coupled to reduced basal and hormone-induced progesterone formation. M1 treatment improved oxygen consumption, cellular respiration, ATP production and mitochondrial proton leak, as well as basal and hormone-induced steroid formation by the TSPO-depleted nG1 cells to levels similar to those seen in wild-type MA-10 cells. nG1 cells also showed disrupted mitochondrial membrane morphology and we found that *Opa1* overexpression in nG1 cells similarly enhanced mitochondrial networks, restored mitochondrial morphology and function.

Conclusion: Taken together these suggest TSPO may play a role in regulating the mitochondrial function. TSPO may exert this regulation by influencing mitochondrial fusion, therefore affecting Leydig cell function. Both M1 treatment and OPA1 transfection enhanced mitochondrial fusion, mitochondrial morphology, and testosterone biosynthesis indicating that steroid formation may be regulated by mitochondrial fusion.

Poster 82

DIFFERENTIAL GENE EXPRESSION ANALYSIS TO ELUCIDATE THE MECHANISM OF TESTICULAR FIBROSIS IN KLINEFELTER SYNDROME

Mark Xu[1,2], Aaron Bradshaw[1,2], Mohammad Darzi[3,4], Abinav Udaiyar[1], Stephen Walker[1], Stanley Kogan[1,2], Stuart Howards[1,2], Thomas McLean[5], Anthony Atala[1,2], Hooman Sadri-Ardekani[1,2]
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Presented By: Mark Xu, MD

Introduction & Objective: Klinefelter's Syndrome (KS) is characterized by widespread testicular fibrosis, germ cell loss, and seminiferous tubule degeneration during puberty. The exact mechanism of this process is unknown. However, alterations of the blood-testis barrier, loss of contractile function in peritubular myoid cells, and derangement in testicular blood flow have all been proposed as possible mechanisms. Macrophages and mast cells are increased in KS testes relative to normal testes, suggesting that inflammation may contribute to the fibrotic process. The objective of this study is to characterize changes in gene expression underlying testicular fibrosis in human KS testes.

Methods: Total RNA was extracted from homogenized, age-matched (patients aged 8-38 years old), paraffin-embedded testicular biopsies from five KS and four control (46XY) patients and analyzed with the NanoString nCounter Fibrosis Panel, which included 770 genes. Raw data was analyzed through R-based software. Change in gene expression was significant if the absolute fold change was > 1.4 or < 0.7 with a p-value < 0.05. Using Ingenuity Pathways Analysis (IPA) software, we analyzed the identified up- and downregulated transcripts to map functional activity and gene networks. IPA generated p-values < 0.05 indicated statistically significant, non-random association, and z-scores > 2 and < -2 indicated activation or deactivation of a functional process, respectively. Weighted gene correlation netowrk analysis (WGCNA) was applied to find co-expressed gene modules.

Results: The NanoString analysis found 19 gene differentially expressed in KS testes compared to controls, with 17 upregulated and 2 downregulated. WGCNA identified six modules of co-expressed genes. 14/17 upregulated genes clustered in the "blue" module, which included 109 genes with significant membership ($p < 0.05$). "Blue" module hub genes included PNPLA3, ELOVL6, and EGFR. IPA showed that key functional categories of differentially expressed genes in KS testes were cellular function/maintenance, immunological disease, hematological system development/function, and inflammatory response. The most highly activated functional processes in KS testes involved vascular development and mobilization of immune cells.

Conclusion: This study indicates that the development of testicular fibrosis in Klinefelter's syndrome is a complex process that involves immune cell mobilization and vascular development. Inflammation may contribute to the derangement of the testis microenvironment in KS, leading to hypogonadism and infertility. Further understanding of this process may help prevent or treat testicular dysfunction in KS patients.

Poster 83

SINGLE-CHANNEL ELECTROPHYSIOLOGY OF SPERMATOGONIA AND SERTOLI CELLS FROM JUVENILE MICE

Lina Kenzler, Marc Spehr
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Presented By: Lina Kenzler, BS, MS

Introduction & Objective: The life-long generation of fertile spermatozoa is *the* most fundamental process for male fertility. Spermatogenesis takes place within the mammalian testis in the seminiferous tubules. Their epithelium is composed of somatic Sertoli and developing germ cells. Entering puberty, spermatogonial stem cells undergo a stereotyped sequence of mitotic and meiotic divisions to constantly generate spermatozoa. Due to low innervation of the testis, spermatogenesis relies on multi-directional communication. However, our understanding of testicular cell communication during this highly complex process of cell proliferation, differentiation, and maturation remains incomplete.

Methods: To gain mechanistic insight into seminiferous tubule signaling, we use patch-clamp recordings in acute tubule sections of juvenile C57BL/6J mice. Sertoli cells and spermatogonia are visually identified by diffusion dye loading in whole-cell configuration, before outside-out patches are formed.

Results: Single channel recordings provide evidence for diverse voltage activated currents in both cell types, including a large outward current. Local application of either iberiotoxine (50 nM) or tetraethylammonium (1 mM) suppress the large outward current. Combining pharmacological profiling and single channel analysis, we provide evidence for the large conductance calcium-activated potassium channel (Slo1) as well as additional distinct yet partly overlapping ion channel repertoires in Sertoli cells and spermatogonia.

Conclusion: Together, this research provides insights into the electrophysiological basis of putative communication strategies and signaling mechanisms in both, prepubertal spermatogonia and Sertoli cells. Ongoing experiments aim to elucidate additional mechanisms that regulate germ cell development and might, hence, identify novel players in male (in)fertility.

Poster 84

PROTEIN-PROTEIN INTERACTION ANALYSES INDICATE ADDITIONAL REGULATORY ROLES FOR SRPK1 AND PP1γ DURING SPERM CHROMATIN COMPACTION

Alberto de la Iglesia[1], Mélina Blanco[2], Meritxell Jodar[1,3], Julie Cocquet[*2], Judit Castillo[*1], Rafael Oliva[*1,3]

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Presented By: Alberto de la Iglesia, MS

Introduction & Objective: Among the complex epigenetic landscape regulating the nucleohistone to nucleoprotamine (NH-NP) transition occurring during spermiogenesis, a correct balance of the effectors of protamine phosphorylation is essential for proper DNA protamination and, ultimately, to produce functional spermatozoa. In the testis, two well-established protamine phosphorylation effectors are SRSP protein kinase 1 (SRPK1), phosphorylating protamine 1, and Serine/threonine-protein phosphatase PP1-gamma (PP1γ), dephosphorylating protamine 2. Their deregulation is related with an impaired sperm chromatin structure and male infertility. The objective of the present work is to decipher whether SRPK1 and PP1γ interact with other proteins involved in sperm chromatin remodeling, in order to help unravel this complex epigenetic process.

Methods: Protein immunoprecipitation (IP) against SRPK1 (#14073-1-AP, Proteintech) and PP1γ (#sc-515943, SCBT) was performed on mouse whole testis lysates in triplicates followed by LC-MS/MS bottom-up proteomics (FDR 1%, $p < 0.01$). Candidates highly expressed in postmeiotic cells were sorted out by analyzing testicular scRNA-seq gene expression data using ReproGenomics Viewer (https://rgv.genouest.org/). Protein networks and functions were determined using STRING (https://string-db.org/) and the Gene Ontology Resource (http://geneontology.org/), respectively.

Results: IP against SRPK1 revealed an interactive network of 41 proteins mainly involved in the regulation of mRNA splicing, transport, metabolism and stability. Among the most expressed ones during late spermatogenesis, we identified RNA binding proteins (RBPs) associated to mRNAs of protamine 2 and transition proteins, with a key role in the NH-NP transition. The PP1γ interactive network was independent of SRPK1, with 61 proteins mainly involved in protein dephosphorylation, signal transduction regulation, and RNA binding. Following the same *in silico* pipeline, we found RBPs involved in MIWI-mediated post-transcriptional regulation of spermiogenic mRNAs, and proteins from the R2TP chaperone complex specialized in quaternary folding of splicing machinery and RNA polymerases.

Conclusion: We report for the first time that SRPK1 and PP1γ interact with proteins involved in the regulation of mRNAs directly involved in sperm chromatin compaction, pointing out new regulatory roles of SRPK1 and PP1γ during spermiogenesis. The identification of these protein networks broadens the functional spectrum of both proteins and is a first step to identifying biomarkers and molecular targets useful to address male reproductive health problems. Work supported by "Ministerio de Ciencia e Innovación" (PI16/00346, PI20/00936 to RO; FI17/00224, MV20/00026 to AI) and by ANR CHROMATOZOA to JCo.

Poster 85

MULTI-OMIC ANALYSIS OF RAT SPERMATOGENESIS

Eoin Whelan[1], Fan Yang[1,2], John Swain[1], Avarbock Mary[1], Antonia Rotolo[1], Elise Krespen[1], Daniel Beiting[1], Ralph Brinster[1]
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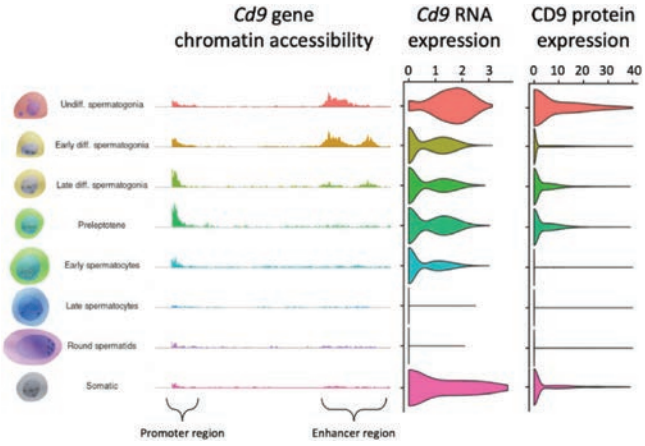
Presented By: Eoin Whelan, BS, PhD

Introduction & Objective: Spermatogonial stem cells form the basis of spermatogenesis, differentiating into all germ cell types including mature spermatozoa. Dramatic changes in chromatin organization accompany alterations in RNA expression and protein levels throughout this process. The rat is a crucial model in male germ cell biology, and the objective of this work was to provide a comprehensive multi-omic map of spermatogenesis, as a prerequisite to understanding the regulation of male germ cell development.

Methods: Sprague-Dawley rat testis cells were dissociated by enzymatic digestion, and single-cell RNA-sequencing (scRNA-seq) was performed jointly with barcode-tagged antibodies (CITE-seq, n = 4) on whole cells, and also in nuclei alongside simultaneous assays for transposase-accessible chromatin (scRNA-seq + scATAC-seq, n = 2). For each replicate, two encapsulations were performed: unselected cells and EpCAM+ selected to enrich for early germ cell stages. Integrated bioinformatic analysis was performed on all samples to evaluate correlations between datasets within each individual cluster. Novel potential spermatogonial stem cell markers were investigated through transplantation assays of rat germ cells into nude mice and further validated via immunohistochemistry and RNA in situ hybridization.

Results: Matched scRNA-seq and scATAC-seq datasets provided a map of male germ cell differentiation, with unique transcriptome and chromatin patterns defining each stage, revealing gene-specific relationships between promoter accessibility, regulatory elements and RNA expression along with the protein product for select genes such as *Cd9* (Figure 1). Rat stem cells appeared to be marked by *Etv5* and *Ret* but not by *Id4*, unlike in mouse where *Id4* is a key transcription factor. Importantly, novel putative stem cell surface markers were identified, including *F3*, *Pdcd1lg2* and *Anpep*, that were associated with robust and specific RNA expression in rat stem cells, but chromatin accessibility and surface protein expression for these genes persisted into differentiating spermatogonia. The same stem cell markers were further validated by transplantation assay in vivo.

Conclusion: Our work provides the first map of chromatin, RNA and protein relationships in rat spermatogenesis. By employing single-cell multi-omic analysis, we profiled regulators and gene networks across each differentiation stage simultaneously, which provided new insight into rat spermatogonial stem cell biology and differentiation. Disconnects between RNA expression and protein underscore the importance of single-cell approaches for both. Our findings form the foundation for future mechanistic studies of germ cell development.



Rat Germ Cell Cd9 Chromatin Accessibility, RNA & Protein

Poster 86

THE EFFECT OF LEPTIN ON TESTICULAR IMMUNE MICROENVIRONMENT (TIME) IS INFLUENCED BY BMI

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Presented By: Alexandra Dullea, MS

Introduction & Objective: Testosterone (T) deficiency affects up to 20% of males. T is produced by Leydig Cells (LC) and LC dysfunction can lead to T deficiency (TD). The development of LC is influenced by paracrine factors released by the testicular micro-environment (TME). Our previous work has demonstrated the role of low dose leptin in the development of human Leydig Stem Cells (LSC) into Adult Leydig Cells (ALC), capable of producing T through desert hedgehog signaling (DHH). Moreover, the impacts of leptin in modulating DHH and LSC differentiation are influenced by patient BMI. Furthermore, we found that low doses of leptin influence the testicular immune micro-environment (TIME) in mice with a normal body habitus. However, the effect of leptin on TIME in different BMI conditions is not fully explored. Therefore, in the present study we evaluated the effects of leptin on the TIME using murine models with different BMI models.

Methods: C57/BL mice were fed with regular, high-fat or low-fat diet for 8 weeks (n = 15 per group). Following the diet, mice in each group were divided into control and treatment sub-groups where treatment sub-groups were injected with two doses of leptin (10 and 100 ug) intraperitoneally for 7 days. After leptin treatment, the mice were euthanized, and blood was collected and was subjected to differential complete blood count (CBC) profiling.

Results: The CBC profiling data was compared between groups. Results showed that in obese mice on low-dose leptin (10ug), white blood cells and platelets were significantly elevated when compared to control mice (regular diet, no leptin treatment) ($p < 0.05$). In the obese, high-dose leptin group (100ug), there was a significant increase in the number of monocytes when compared to control ($p < 0.05$). In the lean, high-dose leptin group, neutrophils were increased, and platelets were decreased when compared to control ($p < 0.05$). Finally, in the lean high-dose leptin group, red blood cells were increased when compared to control ($p < 0.05$). The other parameters such as eosinophils and basophils remained similar between the three groups, irrespective of treatment.

Conclusion: The results demonstrate that low dose leptin has a differential impact on the TIME, as demonstrated by the differences in CBC profiling, which is specifically influenced by BMI. Future studies will explore the influence of BMI on leptin induced changes in LSC differentiation and TIME.

Poster 87

DETERMINATION OF DIAGNOSTIC MOLECULAR MARKERS IN NON-OBSTRUCTIVE AZOOSPERMIA-MALE INFERTILITY VIA NEXT-GENERATION SEQUENCING METHODS

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Presented By: Fahim Rajiwate, MBBS

Introduction & Objective: One in six couples are infertile and male factor contributes 49%. Azoospermia especially non obstructive azoospermia (NOA) poses a great clinical challenge for prediction of fertility.The basic understanding of any disease starts from its physiology which is determined by genes.Hence Studying the molecular basis esp.functional genomics of NOA can help in understanding the molecular mechanism of spermatogenesis, discover potential molecules for further clinical research and utility in diagnostics, prognostics, and therapeutics and also identification of target molecules for research towards the development of new male contraceptives

Methods: Testicular biopsies were collected from 18, 7, 2 and 2 donors, respectively, with Non-Obstructive Azoospermia, Obstructive Azoospermia, Congenital Bilateral Absence of the Vas Deferens and Varicocele, following ethical procedures approved by the Institutional Biosafety Committee at Institute of Bioinformatics & Applied Biotechnology and Ankur-Manipal hospital, Bengaluru. The samples were stored in RNAsolution(Ambion,cat # AM7024) with RNA extraction by RiboPure kit(AM1924).Sequencing and analysis was carried out using TruSeqRNA Library Prep Kit v2,Illumina HiSeq2000. Alignments, identification of transcripts and the chimeric/transplicemolecules, and their quantification were performed by Kallistosoftware. Apart from the standard data analysis procedures for identifying transcripts expressed differently in NOA, a novel scoring system has been applied to rank the transcripts based on the 'Strength of Association' (StA) with NOA. Up- and down-regulated transcripts were hierarchically arranged based on their StA scores.

Results: The study derived the first list of genes and transcripts that are arranged hierarchically based on their StA. Cluster analysis showed that the overall gene expression profiles in NOA were different compared to control samples with normal spermatogenesis - irrespective of sub-types within each set. Based on the 100% consistency of differential expression observed across 18 NOA and 22 control samples, via RNA-seq and/or the RT-qPCR results, 16 mRNAs and three chimeric transcripts promising potential diagnostic markers were identified.

Conclusion: Transcriptomic analysis of the testes with NOA has identified for first time 16 mRNAs and three chimeric transcripts, which are hierarchically listed based on their strength of association, which wouldsuggest the association with spermatogenesis in most cases, particularly those downregulated in NOA. These 19 markers could be used in RT-qPCR assays, individually or in combination to avoid the need for open surgeries for the detection of NOA.

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If we have unintentionally overlooked your contribution, we most sincerely apologize.
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