ANDROLOGY

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Editors-in-Chief:

Ewa Rajpert-De Meyts and Douglas T Carrell

American Society of Andrology

Program Schedule from the 41st American Society of Andrology Annual Meeting

1 – 5, April 2016

New Orleans, Louisiana

SCHEDULE AT A GLANCE

American Society of Andrology 41st Annual Conference
"Old Dogmas, New Ideas: The Changing Landscape in Andrology"
April 1 – 5, 2016
Astor Crowne Plaza

Program Chairs: Arthur L. Burnett II, MD, MBA and Robert S. Viger, PhD
All sessions will be held in ***Grand Ballroom C/D*** unless otherwise noted.
Speakers and times are subject to change

FRIDAY, APRIL 1, 2016

2:00 p.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

6:00 p.m. – 10:00 p.m. **Council Meeting**
Location: Astor III

SATURDAY, APRIL 2, 2016

7:30 a.m. – 7:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

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

8:30 a.m. – 4:30 p.m. ***ASA Basic Science Workshop**
(See page 24 for full program schedule)
**Not CME Accredited*

8:30 a.m. – 5:30 p.m. ***ASA Andrology Lab Workshop**
(See page 25 for full program schedule)
**ABB Laboratory Accredited*

1:00 p.m. – 5:15 p.m. **ASA Special Symposium**
(See page 26 for full program schedule)

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

5:40 p.m. – 6:00 p.m. **Updates from NICHD & NIEHS:**
Stuart B. Moss, PhD
Program Director, Male Reproductive Health Fertility and Infertility Branch, NICHD

6:00 p.m. – 6:20 p.m. ***Distinguished Andrologist Award**
(Introduced by Terry T. Turner)
2016 Recipient: Barry T. Hinton, PhD
**Not CME Accredited*

6:20 p.m. – 7:30 p.m. **EMIL STEINBERGER MEMORIAL LECTURE**
CatSpers: Sperm Specific Calcium Channels Required for Male Fertility
(Introduced by Vassilios Papadopoulos, DPharm, PhD)
David Clapham, MD, PhD
Harvard Medical School

7:30 p.m. – 9:30 p.m. **Welcome Reception**
Location: Astor Ballroom Gallery I/II

SUNDAY, APRIL 3, 2016

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

7:00 a.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

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

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

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
The Changing Paradigm of Prostate Cancer Treatment: Genomics and Beyond
(Introduced by Arthur L. Burnett II, MD, MBA)
Kenneth J. Pienta, MD
Johns Hopkins Hospital

9:00 a.m. – 9:15 a.m. ***Distinguished Service Award**
(Introduced by Sally Perreault Darney, PhD)
2016 Recipient:
Donna L. Vogel, MD, PhD
**Not CME Accredited*

9:15 a.m. - 10:45 a.m. **Symposium I- Somatic Cells of the Testis**
Chair: Lee B. Smith, PhD

Putting the Brakes on Steroidogenesis: Novel Insights into the Fine Regulation of Steroid Hormone Production
Jacques J. Tremblay, PhD
Laval University, Canada

Macrophages: Diverse Players in Testis Biology
Tony De Falco, PhD
University of Cincinnati

Revisiting the Concept of Terminal Differentiation in Adult Sertoli Cells
Peter Stanton, PhD
Hudson Institute of Medical Research, Australia

10:45 a.m. - 11:00 a.m. **Break**

11:00 a.m. - 12:30 p.m. ***Poster Session I**
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

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SCHEDULE AT A GLANCE

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

12:30 p.m. - 2:00 p.m. **MENTORING LUNCHEON**
SPONSORED BY THE DIVERSITY AND TRAINEE AFFAIRS COMMITTEES
Career Guides: The Importance of Finding a Coach, a Mentor, and a Sponsor
Location: Toulouse B (2nd Floor Mezzanine)
George L. Gerton, PhD
Perelman School of Medicine, University of Pennsylvania

2:00 p.m. - 3:30 p.m. **CONCURRENT SESSIONS**
Oral Session I
Location: Grand Ballroom CD
Moderator: Robert S. Viger, PhD

Oral Session II
Location: Grand Ballroom AB
Moderator: Arthur L. Burnett II, MD, MBA

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

3:45 p.m. - 4:30 p.m. **LECTURE I:**
(Introduced by Robert S. Viger, PhD)
Steroid-Binding Proteins-Gatekeepers of Androgen Action
Geoffrey L. Hammond, PhD
The University of British Columbia

4:30 p.m. – 6:30 p.m. ***2nd Annual Trainee Directed Mini-Symposium: "Eyes Wide Open: Understanding the Ins and Outs of Publishing"**
(See page 22 for full program schedule)
**Not CME Accredited*

6:30 p.m. – 8:30 p.m. **Trainee Forum and Mixer**
Location: Grand Gallery
(All Trainee Travel and Onsite Poster Awards (Outstanding Trainee Investigator, Anna Steinberger Award, and Trainee Merrit Awards) will be distributed and celebrated at this event)

MONDAY, APRIL 4, 2016

7:00 a.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

7:00 a.m. – 3:30 p.m. **Exhibit Hall Open**
Location: Astor Ballroom

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

7:00 a.m. – 8:00 a.m. **Industry Satellite Symposium Breakfast**
Location: Toulouse B

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Exploring the Genetic and Genomic Basis of Male Reproductive Defects
(Introduction: Sarah Kimmins, PhD)
Dolores J. Lamb, PhD, HCLD
Baylor College of Medicine

9:00 a.m. – 9:15 a.m. ***Matthew P. Hardy Young Andrologist Award**
(Introduced by Moria K. O'Bryan, PhD)
2016 Recipient: Lee B. Smith, PhD
**Not CME Accredited*

9:15 a.m. - 10:15 a.m. **Symposium II/ Debate**
Moderator: Kirk Lo, MD, FRCSC

Fertility Preservation in Adolescent Klinefelter Patients: the Case (or not) for Early Intervention
Darius A. Paduch, MD, PhD
Weill Medical College of Cornell University

Nathalie Rives
CHU Hôpitaux de Rouen - CHU Charles Nicolle, France

10:15 a.m. - 10:30 a.m. **Break**

10:30 a.m. - 11:15 a.m. **DIVERSITY LECTURE**
My Search for the Reasons for Health Disparities: What I Have Learned and What I Haven't
(Introduced by: Maria Christina W. Avellar, PhD)
Thomas Laveist, PhD
George Washington University

11:15 a.m. - 12:30 p.m. ***Poster Session II**
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

12:30 p.m. - 1:45 p.m. **Lunch On Own**

12:30 p.m. - 1:45 p.m. **WOMEN IN ANDROLOGY LUNCHEON**
Engaging Basic Scientists in Translational Research
Location: Toulouse B (2nd Floor Mezzanine)
Dolores J. Lamb, PhD, HCLD
Baylor College of Medicine

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SCHEDULE AT A GLANCE

12:30 p.m. – 1:45 p.m. **Editorial Board Luncheon**
Location: Toulouse A (2nd Floor Mezzanine)

1:45 p.m. - 3:15 p.m. **SYMPOSIUM III- Novel Therapies in Andrology**
Moderator: John K. Amory, MD, MPH

Use of an Antimicrobial Peptide, LL 37, as a Vaginal Contraceptive
Nongnuj Tanphaichitr, PhD
University of Ottawa

Ghrelin Hormone in Wound Healing and Prevention of Scarring in the Post-operative Setting of Abdominal and Testicular Surgery
Kathleen Hwang, MD
The Warren Alpert Medical School of Brown University

Therapeutic Potential of Transplanted Immune Privilege Sertoli Cells: Unexpected Revelations about Sertoli Cell Dogmas
Jannette Dufour, PhD
Texas Tech University Health Sciences Center

3:15 p.m. - 3:30 p.m. **Break**

3:30 p.m. - 4:15 p.m. **LECTURE II**
(Introduced by Janice Bailey, PhD)
Environmental Programming of the Epigenome in Paternal Reproductive Health
Sarah Kimmins, PhD
McGill University

4:15 p.m. - 5:00 p.m. **LECTURE III**
(Introduced by Larry Lipshultz, MD)
The Role of the Urologist in the Era of IVF/ICSI
Alexander W. Pastuszak, MD, PhD
Baylor College of Medicine

5:00 p.m. - 6:00 p.m. **ASA Business Meeting**

7:00 p.m. – 10:00 p.m. ***Annual Banquet**
Location: Paddlewheeler Creole Queen
1 Poydras Street
New Orleans, LA 70130
Located in Spanish Plaza behind Hilton Riverside and next to Outlet Collection at the Riverwalk

7:00 p.m. - 8:00 p.m. Boarding
8:00 p.m. - 10:00 p.m. Cruising
(Boat will leave dock and return)

Shuttle bus to boat will leave at 6:45 p.m. from the Astor Crowne Plaza Canal Street Entrance and return after the event at 10:00 p.m.

TUESDAY, APRIL 5, 2016

7:00 a.m. – 8:00 a.m. **2017 Program Committee Meeting**
Location: St. Ann (2nd Floor Mezzanine)

7:00 a.m. – 12:30 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

7:00 a.m. – 8:00 a.m. **Continental Breakfast**
Location: Grand Gallery

8:00 a.m. - 9:30 a.m. **SYMPOSIUM IV - Germ Cell/ Spermatogonial Stem Cell Biology**
Moderator: Kyle Orwig, PhD

Defining the Mechanisms Regulation Spermatogonial Differentiation
Chris Geyer, PhD
Brody School of Medicine at ECU

Promoting Recombination in Mouse Meiosis
Sophie La Salle, PhD
Midwestern University

Preservation of Fertility through Transplantation of Germ Cells and Grafting of Testicular Tissue
Ali Honaramooz, DVM, PhD
University of Saskatchewan

9:30 a.m. - 9:45 a.m. **Break**

9:45 a.m. - 10:45 a.m. **INTERNATIONAL LECTURE**
Clinical Markers of Gonadal Function in Prepuberty and Puberty
(Introduced by Patricia Cuasnicu, PhD)
Rodolfo A. Rey, MD, PhD
Hospital de Niños Ricardo Gutiérrez, Buenos Aires

10:45 a.m. - 12:15 p.m. **SYMPOSIUM V - Male Reproductive Tract/Sperm Biology**
Moderator: Sylvie Breton, PhD

Exploring the Role of Macrophages and Dendritic Cells in the Epididymis
Nicolas Da Silva, PhD
Massachusetts General Hospital

Y Chromosome and Spermatogenesis
Monika A. Ward, PhD
University of Hawaii

"Breaking" News from Spermatids
Guylain Boissonneault, PhD
Universite de Sherbrooke, Canada

MEETING ADJOURNED

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TABLE OF CONTENTS

Schedule at a Glance 1

President's Welcome 5

Past Presidents 5

ASA Officers 7

General Meeting Information 8

Message from the Program Co-Chairs 10

Emil Steinberger Memorial Lecture Award 11

AUA Lecture 12

Outstanding Trainee Investigator Award 12

Distinguished Andrologist Award 13

Distinguished Service Award 14

Matthew P. Hardy Young Andrologist Award 15

Thank You to Donors & Sponsors 16

Educational Needs & Objectives 18

Accreditation Statement 19

Schedule of Events 20

Speaker Abstracts 27

Oral Abstracts 35

Poster Session I 41

Poster Session II 52

Index of Abstract Authors 62

Abstract Full Text 67

Committee Listing 130

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PRESIDENT'S WELCOME

Dear Colleagues,

Last year we celebrated the 40 years of the American Society of Andrology. While 40 often signifies the beginning of middle age, linked with the beginning of the reduction of testosterone levels in the male (andropause), there is no evidence that our society is "pausing." In contrast, ASA is tackling the numerous important issues that the field of andrology is facing, ranging from molecules to society.

The highlight of the efforts of the society is its annual meeting where basic scientists and clinicians, trainees, staff, and faculty members are coming together to present, listen, and discuss cutting-edge andrology research presented by our members, trainees, and other scientists from all over the world.

Our Program Chairs, Drs. Arthur Burnett and Robert Viger, the Program Committee, ASA Council, and abstract reviewers have spent a lot of time ensuring that what will be presented at the ASA Annual Meeting is timely, new, of high caliber and interest to both fundamental and clinical andrologists. Indeed, they have put together a great lineup of speakers, talks and topics.

Thank you to the chairs of our pre-conference workshops – Dr. Drobnis, Andrology Lab Workshop; Dr. Snyder, Basic Science Workshop; and Drs. Khera and Kohler, Special Symposium. Thank you to all the committee chairs and members who helped to create all the wonderful optional workshops, luncheons, and optional events hosted throughout the ASA Annual Meeting

However, our annual meeting goes beyond the scientific program. The awards committee, chaired by Dr. O'Brien, spent a lot of time debating on the merit of the nominees. The local arrangements committee, chaired by Drs. Sikka and Hellstrom, together with the W.J. Weiser staff have put together a terrific entertainment for our social activities. New Orleans was chosen to be the site of our meeting two years ago by the future meetings committee, chaired by Dr. McCarrey.

I take this opportunity to thank all of the members of the society and W.J. Weiser staff involved in the organization of this 41st Annual Meeting of the ASA, entitled "Old Dogmas, New Ideas: The Changing Landscape in Andrology" to be held at the *bayou*.

I look forward to meeting you there.

Vassilios Papadopoulos, DPharm, PhD
President, American Society of Andrology

PAST PRESIDENTS OF THE AMERICAN SOCIETY OF ANDROLOGY

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1994-1995	Glenn R. Cunningham
1995-1996	Marie-Claire Orgebin-Crist
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1997-1998	Terry T. Turner
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2004-2005	William J. Bremner
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2006-2007	Christina Wang
2007-2008	Terry R. Brown
2008-2009	Wayne J.G. Hellstrom
2009-2010	Dolores J. Lamb
2010-2011	Paul J. Turek
2011-2012	Gail A. Cornwall
2012-2013	Donna L. Vogel
2013-2014	Erwin Goldberg
2014-2015	Jay I. Sandlow

**Deceased*

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MITCHELL J. LANDRIEU, MAYOR

CITY OF NEW ORLEANS

Welcome!

As Mayor of New Orleans, it is my pleasure to welcome the American Society of Andrology to our City.

Thank you for your continued commitment to scientific excellence and service, which are the backbone to strong healthy communities.

I am certain that New Orleans will serve as the perfect destination for your conference and provide the best opportunities for your members. You will experience the warm hospitality and unique culture that New Orleans has to offer.

While you are here, I hope that you will take some time to visit our historic French Quarter, fine restaurants and entertainment venues and absorb the rich culture that makes New Orleans the most authentic and culturally rich destination in America.

Again, thank you for choosing New Orleans for this year's national meeting and I hope you enjoy your stay.

Sincerely,

Mitch

Mitchell J. Landrieu
Mayor, City of New Orleans

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Ewa Rajpert-De Meyts, MD, PhD, DMSc; Copenhagen, Denmark (Editors-In Chief)

Laboratory Science Forum
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Liaison Committee
Cristian O'Flaherty, PhD, DVM; Montreal, QC Canada (Chair)

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Suresh C. Sikka, PhD, HCLD, CC(ABB); New Orleans, LA (Co-Chair)

Membership Committee
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Nominating Committee
Jay I. Sandlow, MD; Milwaukee, WI (Chair)

Program Committee
Arthur L. Burnett II, MD, MBA; Baltimore, MD (Co-Chair)
Robert S. Viger, PhD; Quebec City, QC Canada (Co-Chair)

Public Affairs and Policy Committee
Ajay K. Nangia, MBBS, FACS; Kansas City, KS (Chair)

Special Symposium
Mohit Khera, MD, MPH; Houston, TX (Co-Chair)
Tobias S. Kohler, MD, MPH, FACS; Springfield, IL (Co-Chair)

Testis Workshop
Leslie L. Heckert, PhD; Kansas City, KS (Chair)

Trainee Affairs
Sophie La Salle, PhD; Downers Grove, IL (Chair)
Mara Roth, MD; Seattle, WA (Co-Chair)

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.

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GENERAL MEETING INFORMATION

ABOUT NEW ORLEANS

New Orleans, with its richly mottled old buildings, its sly, sophisticated –sometimes almost disreputable – air, and its Hispanic-Gallic traditions, has more the flavor of an old European capital than an American city. Townhouses in the French Quarter, with their courtyards and carriageways, are thought by some scholars to be related on a small scale to certain Parisian "hotels" – princely urban residences of the 17th and 18th centuries. Visitors particularly remember the decorative cast-iron balconies that cover many of these townhouses like ornamental filigree cages.

European influence is also seen in the city's famous above-ground cemeteries. The practice of interring people in large, richly adorned aboveground tombs dates from the period when New Orleans was under Spanish rule. These hugely popular "cities of the dead" have been, and continue to be, an item of great interest to visitors. Mark Twain, noting that New Orleanians did not have conventional below-ground burials, quipped that "few of the living complain and none of the other."

Attractions

Take a bike ride along the Mississippi River, feed an alligator or stroll through time as you take in the stories behind one of our many historical attractions. New Orleans has so much to offer and caters to every traveler. So whether you're a sports fan, history buff, outdoor adventurer, aspiring chef, or music lover, there's an attraction in New Orleans to suit your every need.

Shopping

New Orleans shopping – from the French Quarter to Magazine Street – offers an array of retail choices. Options include The Shops at Canal Place, Saks Fifth Avenue, Outlet Collection at Riverwalk, as well as hundreds of boutiques, art galleries and antique stores throughout the city. Tax-free shopping offers international visitors even more reasons to experience New Orleans.

Dining/Nightlife

New Orleans offers one of the most incredible – and incredibly diverse –concentrations of exceptional dining and unforgettable cuisine in the world.

The night never has to end in New Orleans since bars and clubs are not required to close. In fact, many say the city is at its best under the glow of a French Quarter street lamp. But to suggest the party starts and ends on Bourbon Street sells the rest of the city short. New Orleans has many distinct neighborhoods that offer nightlife options from incredible live music on Frenchmen Street to intimate cocktails just a neighborhood away. Harrah's Casino caters to the high rollers at heart and the city is home to slick nightclubs where you can dance the night away.

Arts & Culture

New Orleans' Old World roots have created a strong foundation and long-standing appreciation for the arts. Early residents of the city often traveled back to Europe for musical instruction or training in the visual arts, and operatic performances took place in the city as early as 1796.

Today the scene thrives via countless galleries, performance spaces and museums. But perhaps what sets our cultural institutions apart from those of other cities is their ability to embody the joie de vivre (joy of life) so prevalent in New Orleans. So whether it is a Ralph Brennan restaurant in the New Orleans Museum of Art, or Thursday night musical performances at the Ogden Museum of Southern Art, New Orleans' unique culture and love of food, music, and fun blends perfectly with the vibrant arts scene creating experiences you can't find anywhere else.

HOTEL INFORMATION

Astor Crowne Plaza – New Orleans French Quarter
739 Canal Street at Bourbon
New Orleans, LA 70130
Stationed at the intersection of Canal Street and Bourbon Street, Astor Crowne Plaza – New Orleans French Quarter is ideally positioned near the area's biggest and brightest attractions. Walk to the Big Easy's most compelling local points of interest, including Ernest N. Morial Convention Center, Harrah's Casino, the French Market and Riverwalk.

Main: (504) 692-0500
Fax: (504) 962-0503
Website: www.astorneworleans.com

TRAVEL AND TRANSPORTATION

Airport Information

Louis Armstrong New Orleans International Airport is located about 14 miles from the Astor Crowne Plaza – New Orleans French Quarter.

Taxi Cab Services

The airport shuttle is $22 each way or taxi is $33 each way (one or two persons).

Rental Car Information

Avis® Rent-A-Car is the official rental car company for the ASA 41st Annual Conference. For reservations, please call (800) 331-1600, and use the code "J901055" to receive the discounted rates.

Parking

Valet parking is $41 daily plus tax. Self parking is available off site, managed by Premium Parking at 716 Iberville Street, $25 for 24 hours. Visit premiumparking.com for more information.

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GENERAL MEETING INFORMATION

Registration/Information Desk Hours are as follows:

Friday, April 1, 2016	2:00 p.m. – 6:00 p.m.
Saturday, April 2, 2016	7:30 a.m. – 7:00 p.m.
Sunday, April 3, 2016	7:00 a.m. – 6:00 p.m.
Monday, April 4, 2016	7:00 a.m. – 6:00 p.m.
Tuesday, April 5, 2016	7:00 a.m. – 12:30 p.m.

Exhibit Hall Hours are as follows:

Saturday, April 2, 2016	4:00 p.m. – 9:30 p.m.
Sunday, April 3, 2016	7:00 a.m. – 4:00 p.m.
Monday, April 4, 2016	7:00 a.m. – 3:30 p.m.

OPTIONAL WORKSHOPS/SYMPOSIA

Andrology Lab Workshop

"Restoring Strict Morphology Relevance: A Consensus Workshop"
Date: Saturday, April 2, 2016
Time: 8:30 a.m. – 5:30 p.m.
Cost: $200.00 for Attendees (Member/Non-Member). Limited Registration to the first 50 attendees. Please note the Lab Science Forum Lunch is included in ALW registration fee.

ASA Basic Science Workshop

"ANDRomics: Genomics, Epigenomics, and Proteomics of Male Fertility"
Date: Saturday, April 2, 2016
Time: 8:30 a.m. – 4:30 p.m.
Cost: $40.00 for Attendees (Member/Non-Member).

ASA Special Symposium

"What's New in Men's Health"
Date: Saturday, April 2, 2016
Time: 1:00 p.m. – 5:15 p.m.
Cost: Included in the ASA Annual Conference Registration fees, however requires RSVP.

ASA Trainee Directed Mini-Symposium

"Eyes Wide Open: Understanding the Ins and Outs of Publishing"
Sunday, April 3, 2016
Time: 4:30 p.m. – 6:30 p.m.
Cost: Included in the ASA Annual Conference Registration fees, however requires RSVP.

SPECIAL EVENTS

Welcome Reception

Date: Saturday, April 2, 2016
Time: 7:30 p.m. – 9:30 p.m.
Join us for a welcome reception to connect with friends and colleagues. Admission to the reception is included in your ASA meeting registration fee.
Dress: Business casual or casual attire is appropriate
Cost: One ticket included in ASA registration; $25.00 for additional tickets.

Trainee Forum and Mixer

Date: Sunday, April 3, 2016
Time: 6:30 p.m. – 8:30 p.m.
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.

Annual Banquet

Date: Monday, April 4, 2016
Time: 7:00 p.m. – 8:00 p.m. Boarding
8:00 p.m. – 10:00 p.m. Cruising
Location: Paddlewheeler Creole Queen
Cost: $75.00 for Attendees (Member/Non-Member), $35.00 for Trainees.
Join ASA as we step back into the glamour and romance of the riverboat era with a cruise aboard the Paddlewheeler Creole Queen! Experience the sounds of jazz while enjoying a lavish creole buffet in elegantly appointed dining rooms. After dinner partake in a little dancing or enjoy a classic New Orleans cocktail on the promenade deck viewing as the city skyline slips under a canopy of stars. Ticket includes dinner and entertainment.

Buses leave at 6:45 p.m. from the Astor Crowne Plaza Canal Street Entrance. Return buses will leave after the event at 10:00 p.m.

Laboratory Science Forum Luncheon

"Using Immunodetection Techniques for Assessing Fertility"
Date: Saturday, April 2, 2016
Time: 12:00 p.m. – 1:30 p.m.
Speaker: Marie-Claude Hofmann, PhD; Univ. of Texas - MD Anderson Cancer Center
Cost: $35.00 for Attendees (Member/Non-Member).
Please note if you are registered for the Andrology Lab Science Workshop, one ticket for the Laboratory Science Forum Lunch is included in registration.

Mentoring Luncheon

Sponsored by the Diversity and Trainee Affairs Committees
"Career Guides: The Importance of Finding a Coach, a Mentor, and a Sponsor"
Date: Sunday, April 3, 2016
Time: 12:30 p.m. – 2:00 p.m.
Speaker: George Gerton, PhD; Perlman School of Med At the Univ. of PA
Cost: $10.00 for Trainees, $35.00 for Attendees (Member/Non-Member).

Women in Andrology Luncheon and Discussion

"Engaging Basic Scientists in Translational Research"
Date: Monday, April 4, 2016
Time: 12:30 p.m. – 1:45 p.m.
Speaker: Dolores J. Lamb, PhD, HCLD; Baylor College of Medicine
Host: Marie-Claude Hofmann, PhD; Univ. of Texas - MD Anderson Cancer Center
Cost: $25.00 for Trainees, $35.00 for Attendees (Member/Non-Member).

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MESSAGE FROM THE PROGRAM CO-CHAIRS

Friends and Colleagues,

Welcome to the 41st Annual Meeting of the American Society of Andrology!

This year's theme for the ASA meeting is "Old Dogmas, New Ideas: The Changing Landscape in Andrology," which signifies the constant evolution of our field. The advancement of scientific knowledge in testis biology, steroidogenesis and spermatogenesis, genetics, immune biology, cancer biology, and environmental biology among diverse topic areas of our field, position us to develop novel therapies relevant for preserving male reproductive health, fertility, sexual function, and men's health overall. Accordingly, this meeting is designed to showcase the latest science in andrology that has significantly changed, is changing, or has the potential to change our thinking and understanding of our field.

We are indebted to the experts and thought leaders representing disciplines of urology, endocrinology, clinical andrology, genetics, reproductive medicine and reproductive biology, whom we have enlisted as lecturers, debaters, and session moderators for this program. We continue the tradition of the Society's meeting, which fosters scientific knowledge exchange and interaction of all colleagues – basic scientists and clinician-scientists, seasoned investigators and young researchers, long-time ASA members and new members of our society – whose contributions are sincerely appreciated. We wish to emphasize the commitment of the Society to welcome and encourage young trainees in our field. To that end, take note that this year's program features the trainee symposium within the course of the general meeting, which then serves also as a lead-in to the trainee mixer. We hope that you will find time to attend these events and come support our trainees.

We extend our heartfelt gratitude to the ASA President Vassilios Papadopoulos for providing us with the opportunity to co-chair the program for the 2016 Annual Meeting. We are honored and delighted to have been able to serve our Society in this capacity. We also thank the Program Committee members who have supported us in all aspects of planning and coordinating the meeting program. Lastly, we acknowledge the support of the ASA Executive Office and W.J. Weiser and Associates in this endeavor.

The success of this meeting owes to the efforts of many individuals, to whom we are most grateful.

We hope you enjoy the meeting and your time in New Orleans!

Robert S. Viger, PhD Arthur L. Burnett II, MD, MBA

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EMIL STEINBERGER MEMORIAL LECTURE AWARD

David E. Clapham, MD, PhD
Harvard Medical School

David E. Clapham received his MD and PhD from Emory University. He completed his residency in internal medicine at Boston's Brigham and Women's Hospital, and his postdoctoral research fellowship at the Max Planck Institute in Göttingen, Germany. Dr. Clapham is a Howard Hughes Medical Institute Investigator and member of the American Academy of Arts and Sciences (2000) and the National Academy of Sciences (2006), and fellow of the American Association for the Advancement of Science (2008). He is currently the Aldo R. Castañeda Professor of Cardiovascular Research, Professor of Neurobiology, and Professor of Pediatrics at Harvard Medical School. In addition to these remarkable distinctions, Dr. Clapham has been the recipient of numerous other prestigious awards and prizes. The Arnall Patz Lifetime Achievement Award from Emory University Medical Alumni Association (2009), Mayo Clinic Distinguished Alumni Award (2010), and the Harvard Medical School William Silen Lifetime Achievement in Mentoring Award (2013) are among his many honors. His research has been published in the most distinguished scientific journals, including *Cell, Science, and Nature*. In 2014, he was named by Thompson Reuters as among the top 1% of researchers for most cited documents in their field. He is also well recognized for his extensive participation in graduate and medical education training and his contributions to the scientific community where he has served as a member of the editorial boards or as an associate editor for journals such as *Neuron, Cell, Science, Nature Communications* and *PNAS*. Dr. Clapham is an internationally recognized expert on ion channels and how they mediate calcium entry across the plasma membrane. His novel studies led to the identification of spermatozoa-specific ion channels called CatSpers, their function in male fertility, and their role as potential targets for male contraception or some forms of male infertility.

SERONO LECTURESHIP RECIPIENTS

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

ASA LECTURESHIP RECIPIENTS

2004	Judith Kimble
2005	David Page
2006	Gerald P. Schatten
2007	Rudolf Jaenisch
2008	Haifan Lin
2009	Blanche Capel

EMIL STEINBERGER MEMORIAL LECTURE RECIPIENTS

2010	Andrew Sinclair
2011	Leendert Looijenga
2012	William F. Crowley, Jr.
2013	Deborah O'Brien
2014	Rudolf Jaenisch
2015	Marisa S. Bartolomei

The Emil Steinberger Memorial Lecture Award is sponsored by the Emil Steinberger Endowment Fund.

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AUA LECTURE

Kenneth J. Pienta, MD
Johns Hopkins University School of Medicine

Kenneth J. Pienta received his BA and MD degrees at the Johns Hopkins University in 1983 and 1986, respectively, followed by completion of internship and residency in internal medicine at the University of Chicago Hospitals & Clinics, and then fellowship in oncology at the Johns Hopkins University School of Medicine. In 1991, he was appointed Assistant Professor of Medicine in the Division of Hematology and Oncology with a co-appointment in Molecular Biology and Genetics at the Wayne State University School of Medicine, with an additional promotion to Associate Professor at that institution in 1994. In 1994, he was appointed Associate Professor of Internal Medicine at the University of Michigan Medical School with promotion to Professor of Internal Medicine and Surgery in 1998, and then Professor of Urology in 2001 all at that same institution. In 2013, he was recruited back to the Johns Hopkins University School of Medicine as Professor of Urology, Oncology, Pharmacology and Molecular Sciences, and Chemical and Biomolecular Engineering in 2013. At that time, he also became Director of the Urology Research Laboratories at the Brady Urological Institute and Program Leader for the Prostate Cancer Program of the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University School of Medicine. Among many awards, Dr. Pienta was honored in 2011 with the Taubman Research Scholar at the University of Michigan, and in 2014 with the Donald S. Coffey Award of the Society of Basic Urologic Research. Between 1995 and 2013, Dr. Pienta served as the Director of the Prostate Specialized Program of Research Excellence (SPORE) at the University of Michigan, charged with organizing and administering a proven translational research program that successfully incorporated basic research, agent development, and clinical application in prostate cancer biology. Currently, his research involves defining tumor microenvironments and how they contribute to tumorigenesis and metastasis.

OUTSTANDING TRAINEE INVESTIGATOR AWARD

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

The recipient of the 2016 Outstanding Trainee Investigator Award will be announced during the Annual Business Meeting on Monday, April 4, 2016, at 5:00 p.m.

NEW INVESTIGATOR AWARD RECIPIENTS

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

OUTSTANDING TRAINEE INVESTIGATOR AWARD RECIPIENTS

2004	Darius Paduch
2005	Tara Barton
2006	Liwei Huang
2007	Steve Tardif
2008	Duangporn Jamsai
2009	Catherine Itman
2010	Michael Elliott
2011	Matthew Marcello
2012	Andrew Major
2013	Mary Samplaski
2014	Andrew Midzak
2015	Qi Fu

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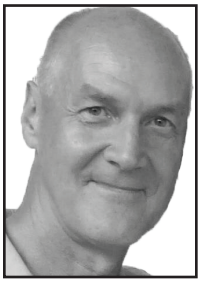
DISTINGUISHED ANDROLOGIST AWARD

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



Barry T. Hinton, PhD
University of Virginia

Barry T. Hinton is currently a professor in the Department of Cell Biology at the University of Virginia.

Born in England, Dr. Hinton received his BSc in pharmacology and biochemistry at the University of East London. After, he worked at the Max Planck Institute for Biophysics in Frankfurt, Germany, as a postgraduate scholar. He then completed his PhD under the mentorship of Dr. Brian P. Setchell at The Babraham Institute in Cambridge. Dr. Hinton then obtained a prestigious Rockefeller Foundation Postdoctoral Fellowship where he worked with Dr. Stuart Howards in the Department of Urology at the University of Virginia.

Upon completion of this fellowship, Dr. Hinton moved to the Department of Cell Biology at the University of Virginia. His initial research focus was on the measurement of the organic solutes in epididymal luminal fluid but then moved onto addressing the mechanisms by which the luminal fluid microenvironment was formed, the regulation of gene expression, and the regulation of initial segment function, a key region involved in male fertility, by testicular lumicrine factors. Currently, Dr. Hinton's research focuses on Wolffian duct morphogenesis: examining the mechanisms by which the duct elongates and coils during development.

Dr. Hinton's research accomplishments include speaking at several national and international meetings. He is a member of NIH Study Sections, and has been awarded ASA's Young Andrologist Award. Further, Dr. Hinton has been awarded the best published paper in the *Journal of Andrology*. In recognition of his service to ASA as secretary, past president, chair, and member of numerous committees, he was awarded the Distinguished Service Award. He is also the current Director of Section Three of the Frontiers in Reproduction Course held each year at the MBL labs, Wood's Hole.

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

The Distinguished Andrologist Award is sponsored by the Eugenia Rosemberg Endowment Fund

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DISTINGUISHED SERVICE AWARD

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

Donna L. Vogel, MD, PhD

Donna L. Vogel, MD, PhD graduated from Bryn Mawr College, and the Medical Scientist Training Program at the Albert Einstein College of Medicine. Her PhD is in developmental biology, and her clinical subspecialty is endocrinology. She worked at NIH for 25 years, initially as a clinical fellow conducting clinical and basic research on male infertility. While managing NICHD's Reproductive Medicine grant program for 13 years, Dr. Vogel realized she most enjoyed working with trainees and early career scientists. In 2001, she became the first Director of an Office for postdocs at NCI. In 2007, she joined Johns Hopkins Medical Institutions as Director of the Professional Development Office, providing courses, workshops, and guidance on transitioning to independent careers in science. She has organized scientific meetings, published research and administrative papers and book chapters, and has received awards from NIH, the US Public Health Service, Hopkins, and professional and community groups.

An ASA member since 1983, Dr. Vogel is past president of the Society (2012–13) and Chair of Women in Andrology (1994–96). She was PI of the conference grant for several years and wrote a successful renewal. She served on Executive Council and on committees including Awards, Student Affairs, Nominating (Chair), Constitution and Bylaws (Chair), Membership, and Trainee Affairs (for longer than she cares to remember), and Endowment & Development. She has an active interest in ASA activities concerning women, trainees, and the diversity community.

Dr. Vogel retired in 2015, and continues to speak and advise on scientific careers and professional skill-building.

DISTINGUISHED SERVICE AWARD RECIPIENTS

1994	C. Alvin Paulsen
1995	Andrzej Bartke
1996	Philip Troen
1997	Marie-Claire Orgebin-Crist
1998	Rupert P. Amann
1999	David W. Hamilton
2000	Bernard Robaire
2001	Gail S. Prins
2002	Terry T. Turner
2003	Arnold M. Belker
2004	J. Lisa Tenover
2005	Barry Hinton
2006	Barry Zirkin
2007	Sally Perreault Darney
2008	Matthew P. Hardy
2009	Erwin Goldberg
2010	Joel L. Marmar
2011	Christina Wang
2012	Terry R. Brown
2013	Rex A. Hess
2014	Susan Rothmann
2015	Steven M. Schrader

Distinguished Service Award is sponsored by the American Society of Andrology.

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MATTHEW P. HARDY YOUNG ANDROLOGIST AWARD

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.

Lee B. Smith, BSc, PhD, FRSB

Professor Lee Smith completed a BSc in genetics at the University of Leicester in the United Kingdom, and a PhD in molecular genetics at the University of Warwick in the United Kingdom, before completing postdoctoral training at the Medical Research Council (MRC), Mammalian Genetics Unit in Harwell, and the University of Oxford, where he focused upon the generation and analysis of knockout mice affecting sex determination.

Prof. Smith moved to Edinburgh in 2006 to develop his own team at the MRC Centre for Reproductive Health. His research focuses upon genetic and endocrine impacts on male reproductive function. Recent efforts include a complete dissection of the role for testosterone in male fertility, resulting in more than 50 publications in high-ranked journals in the past five years.

Prof. Smith's success led to rapid promotion through the academic ranks culminating in the award of the University of Edinburgh, Chair of Genetic Endocrinology (Tenured Full Professor), at the age of 37.

He currently holds competitive research grants totaling more than $17M, including the incredibly prestigious award of a $3.4M MRC Programme Grant to a sole investigator. This successful research program has recently been endorsed for the next five years ($3.1M MRC Programme Grant 2016-2021).

Prof. Smith's world-leading reputation is further reflected in the more than 25 recent invitations to speak at international conferences, including lectures at both the European and American testis workshops. He was elected Fellow of the Royal Society of Biology (FRSB) in 2014, and has recently been appointment as the youngest member of the MRC Population and Systems Medicine Board.

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
2002	Christopher L.R. Barratt
2003	Joanna E. Ellington
2004	Kate Loveland
2005	Janice Bailey
2006	Janice P. Evans
2007	John K. Amory
2008	Moira K. O'Bryan
2009	Michael A. Palladino
2010	Peter Liu
2011	Humphrey Yao
2012	Wei Yan
2013	Jacques J. Tremblay
2014	Sarah Kimmins
2015	Jon M. Oatley, PhD

The Matthew P. Hardy Young Andrologist Award is sponsored by the Matthew P. Hardy Endowment Fund

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Please Be Sure to Attend the Following Industry Satellite Symposium

MONDAY, APRIL 4, 2016

7:00 a.m. - 8:00 a.m.	**Industry Satellite Symposium Breakfast** *Location: Toulouse B* ***"Treatment for Adult Male Hypogonadism Due to an Associated Medical Condition"*** **Chhay Tay, MD** *Humble, Texas*

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EDUCATIONAL NEEDS AND OBJECTIVES

41st Annual ASA Conference
"Old Dogmas, New Ideas:
The Changing Landscape in Andrology"

Educational Needs:
The field of andrology is constantly evolving with the advancement of scientific knowledge surrounding all aspects of male reproductive and sexual health. The science of male reproduction is grounded in scientific advances being made in testis biology, steroidogenesis and spermatogenesis. New knowledge in genetics, immune biology, and environmental biology are all relevant in understanding and preserving the health of the male reproductive tract. Such advances promise the development of novel therapies for preserving testicular function and fertility. Emerging scientific tools such as genomics may impact the understanding of male reproductive tract organs and direct therapeutic endeavors designed to preserve male reproductive tract health. Interactions between basic scientists and clinicians also remains an important objective to bringing forward scientific developments in the field.

The 41st Annual Conference of the American Society of Andrology is designed to showcase speakers and topics that have significantly changed, are changing, or have the potential to change our thinking and understanding of both basic and clinical andrology. Experts and thought leaders in fields of urology, endocrinology, clinical andrology, genetics, reproductive medicine, and reproductive biology will convene to present the latest developments in the molecular, cellular, and systems biologic foundations of male reproductive tract health and function.

Educational Objectives:
At the conclusion of the ASA 41st Annual Conference, attendees should be able to:

1. Recognize the role of sperm-specific calcium channels in normal sperm function as well as potential drug targets for regulating male fertility.
2. Explain the impact of prostate cancer tumor sequencing in defining prostate tumorigenesis and directing the treatment of advanced prostate cancer.
3. Identify the roles of somatic cells (Leydig, macrophages, and Sertoli cells) in testis development and function.
4. Describe the roles of steroid carrier proteins in controlling hormone bioavailability and their potential diagnostic value in endocrinology and clinical andrology.
5. Describe and understand the options for the management of fertility issues in adolescent Klinefelter patients.
6. Explain the mechanism of action of antimicrobial peptides as a sperm-targeting, vaginal contraceptive.
7. Recognize the potential therapeutic role of ghrelin in promoting wound healing and preventing scarring in post-operative abdominal and testicular surgery.
8. Recognize the potential therapeutic role of testicular Sertoli cells in providing immunoprotection following transplantation surgeries.
9. Recognize that the paternal epigenome is a sensitive target to environmental exposures that may be transmitted between generations.
10. Describe the role of the urologist in performing a complete male evaluation in the setting of infertility.
11. Describe the mechanisms that regulate spermatogonial (male germ cell) differentiation.
12. Describe meiotic recombination events in male germ cell meiosis.
13. Recognize and understand the potential value of testicular transplantation and xenografting techniques in fertility preservation and restoration.
14. Explain the utility of biomarkers of prepubertal testicular function in the assessment of male hypogonadism in boys.
15. Describe the arrangement of dendritic cells and macrophages in the epididymis that comprises a mucosal immune system.
16. Recognize the roles of genes encoded on the Y chromosome that are involved in meiotic progression.
17. Explain the significance and mechanisms of DNA fragmentation in spermatogenesis.

ASA Special Symposium:
"What's New in Men's Health"

Educational Needs:
Clinicians should be educated on the latest outcome data on the various treatment approaches to BPH and know the true effects of testosterone levels on BPH/LUTS.

Surgeons should be educated on the latest outcome data on the various surgical approaches used to correct Peyronie's disease.

Surgical learning is a lifelong process that is aided by collaboration and interaction with colleagues. A forum for discussion of technique and procedures helps disseminate ideas and improve patient outcomes.

Clinicians need to be aware of the effects of lifestyle change as pertains to sexual medicine. Specifically, how do environmental factors, smoking, exercise and diet affect sexual health and function from both a clinical and biochemical perspective.

Educational Objectives:
At the conclusion of the ASA Special Symposium: "What's New in Men's Health," attendees should be able to:

1. Recognize the importance of patient selection and careful counseling on expectations before surgery for correction of BPH/LUTS.
2. Explain the various surgical and non surgical approaches for BPH/LUTS.
3. Describe the mechanisms of Testosterone effect on prostate physiology.
4. Explain the importance of patient selection and careful counseling on expectations before surgery for correction of Peyronie's Disease.
5. Identify the various approaches to penile plication and relative advantages/risks of these approaches.
6. Review various approaches to common steps in penile implant placement, including positioning of the patient, selection of infection prophylaxis, access to the corporal bodies, means of placing various implant components and post-operative care.
7. Evaluate best practices and alternatives for a better understanding on how to best perform implant surgery.
8. Describe both biochemical and clinical effects of smoking and smoking cessation, poor and healthy dietary choices, body weight, and exercise on erectile and prostate function.

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CONTINUING MEDICAL EDUCATION CREDIT INFORMATION

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

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SCHEDULE OF EVENTS

American Society of Andrology 41st Annual Conference
"Old Dogmas, New Ideas: The Changing Landscape in Andrology"
April 1 – 5, 2016
Astor Crowne Plaza

Program Chairs: Arthur L. Burnett II, MD, MBA and Robert S. Viger, PhD
All sessions will be held in ***Grand Ballroom C/D*** unless otherwise noted.

FRIDAY, APRIL 1, 2016

2:00 p.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

6:00 p.m. – 10:00 p.m. **Council Meeting**
Location: Astor III

SATURDAY, APRIL 2, 2016

7:30 a.m. – 7:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

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

8:30 a.m. – 4:30 p.m. ***ASA Basic Science Workshop**
(See page 24 for full program schedule)
**Not CME Accredited*

8:30 a.m. – 5:30 p.m. ***ASA Andrology Lab Workshop**
(See page 25 for full program schedule)
ABB Laboratory Accredited

1:00 p.m. – 5:15 p.m. **ASA Special Symposium**
(See page 26 for full program schedule)

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

5:40 p.m. – 6:00 p.m. **Updates from NICHD & NIEHS:**
Stuart B. Moss, PhD
Program Director, Male Reproductive Health Fertility and Infertility Branch, NICHD

6:00 p.m. – 6:20 p.m. ***Distinguished Andrologist Award**
(Introduced by Terry T. Turner)
2016 Recipient: Barry T. Hinton, PhD
**Not CME Accredited*

6:20 p.m. – 7:30 p.m. **EMIL STEINBERGER MEMORIAL LECTURE**
CatSpers: Sperm Specific Calcium Channels Required for Male Fertility
(Introduced by Vassilios Papadopoulos, DPharm, PhD)
David Clapham, MD, PhD
Harvard Medical School

7:30 p.m. – 9:30 p.m. **Welcome Reception**
Location: Astor Ballroom Gallery I/II

SUNDAY, APRIL 3, 2016

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

7:00 a.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

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

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

8:00 a.m. – 9:00 a.m. **AUA LECTURE**
The Changing Paradigm of Prostate Cancer Treatment: Genomics and Beyond
(Introduced by Arthur L. Burnett II, MD, MBA)
Kenneth J. Pienta, MD
Johns Hopkins Hospital

9:00 a.m. – 9:15 a.m. ***Distinguished Service Award**
(Introduced by Sally Perreault Darney, PhD)
2016 Recipient:
Donna L. Vogel, MD, PhD
**Not CME Accredited*

9:15 a.m. - 10:45 a.m. **Symposium I- Somatic Cells of the Testis**
Chair: Lee B. Smith, PhD

Putting the Brakes on Steroidogenesis: Novel Insights into the Fine Regulation of Steroid Hormone Production
Jacques J. Tremblay, PhD
Laval University, Canada

Macrophages: Diverse Players in Testis Biology
Tony De Falco, PhD
University of Cincinnati

Revisiting the Concept of Terminal Differentiation in Adult Sertoli Cells
Peter Stanton, PhD
Hudson Institute of Medical Research, Australia

10:45 a.m. - 11:00 a.m. **Break**

11:00 a.m. - 12:30 p.m. ***Poster Session I**
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

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SCHEDULE OF EVENTS

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

12:30 p.m. - 2:00 p.m. **MENTORING LUNCHEON**
SPONSORED BY THE DIVERSITY AND TRAINEE AFFAIRS COMMITTEES
Career Guides: The Importance of Finding a Coach, a Mentor, and a Sponsor
Location: Toulouse B (2nd Floor Mezzanine)
George L. Gerton, PhD
Perelman School of Medicine, University of Pennsylvania

2:00 p.m. - 3:30 p.m. **CONCURRENT SESSIONS**
Oral Session I
Location: Grand Ballroom CD
Moderator: Robert S. Viger, PhD

2:00 p.m.
SERTOLI CELL GENE THERAPY IN SERTOLI CELL ANDROGEN RECEPTOR KNOCKOUT (SCARKO) MICE
Speaker: Chatchanan Doungkamchan, MD
University of Pittsburgh

2:15 p.m.
GLYCOLYSIS OPTIMIZED CONDITIONS IMPROVE THE REGENERATIVE QUALITY OF SPERMATOGONIAL STEM CELLS DURING LONG-TERM IN VITRO MAINTENANCE
Speaker: Aileen Helsel
Washington State University

2:30 p.m.
THE TYROSINE KINASE FER IS RESPONSIBLE FOR THE CAPACITATION-ASSOCIATED INCREASE IN TYROSINE PHOSPHORYLATION
Speaker: Antonio Alvau, MSc
University of Massachusetts

2:45 p.m.
LACK OF MEMBRANE ESTROGEN RECEPTOR ALPHA LEADS TO SPERM ABNORMALITIES, DECREASED SPERM MOTILITY AND VIABILITY, AND PROGRESSIVE INFERTILITY IN MALE MICE
Speaker: Manjunatha K. Nanjappa, PhD
University of Florida

3:00 p.m.
LONG-TERM BRDU LABELING IDENTIFIES MITOTICALLY ACTIVE ADARK AND APALE SPERMATOGONIA IN ADULT RHESUS MACAQUE TESTES
Speaker: Adetunji Fayomi
University of Pittsburgh

3:15 p.m.
PROTEIN TYROSINE KINASE 7 SIGNALS THROUGH MYOSIN II TO REGULATE PLANAR CELL POLARITY-DEPENDENT TUBULAR MORPHOGENESIS OF THE WOLFFIAN/EPIDIDYMAL DUCT, WHICH IN TURN IS IMPORTANT FOR THE ACQUISITION OF SPERM MOTILITY
Speaker: Bingfang Xu, PhD
University of Virginia

Oral Session II
Location: Grand Ballroom AB
Moderator: Arthur L. Burnett II, MD, MBA

2:00 p.m.
ANDROGEN RECEPTOR DEGRADATION BY SULFORAPHANE ENHANCES THE EFFICACY OF ANTI-ANDROGENS AGAINST AGGRESSIVE PROSTATE CANCER CELLS
Speaker: Namrata Khurana, PhD
Tulane University

2:15 p.m.
CYSTEINE-RICH SECRETORY PROTEIN 3 (CRISP-3) IS A CANDIDATE BIOMARKER OF TESTICULAR DYSFUNCTION IN ADOLESCENTS WITH VARICOCELE
Speaker: Larissa Berloffa Belardin, BSc
Universidade Federal de São Paulo

2:30 p.m.
IFT140 IS A NOVEL CANDIDATE GENE FOR IMPAIRED SPERMATOGENESIS: IDENTIFICATION BY WHOLE EXOME SEQUENCING AND VALIDATION WITH SANGER SEQUENCING
Speaker: Amin S. Herati, MD
Baylor College of Medicine

2:45 p.m.
CASANOVA: A MULTICLASS SUPPORT VECTOR MACHINE MODEL FOR THE CLASSIFICATION OF HUMAN SPERM MOTILITY PATTERNS
Speaker: Summer Goodson, PhD
University of North Carolina

3:00 p.m.
THE EFFECT OF ORCHIECTOMY ON FUNCTIONAL ASPECTS OF SPERM AND OXIDATIVE STRESS OF SEMINAL PLASMA FROM PATIENTS WITH TESTICULAR TUMORS
Speaker: Maria Beatriz Ribeiro De Andrade, BSc
Sao Paulo Federal University

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SCHEDULE OF EVENTS

3:15 p.m.
CONSISTENT DIFFERENCES AMONG MEN IN CAPACITATION TIMING COULD PERSONALIZE IUI/ART
Speaker: Alexander J. Travis, VMD, PhD
Cornell University

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

3:45 p.m. - 4:30 p.m. **LECTURE I:**
(Introduced by Robert S. Viger, PhD)
Steroid-Binding Proteins-Gatekeepers of Androgen Action
Geoffrey L. Hammond, PhD
The University of British Columbia

4:30 p.m. – 6:30 p.m. ***2nd Annual Trainee Directed Mini-Symposium: "Eyes Wide Open: Understanding the Ins and Outs of Publishing"**
Location: Grand Ballroom C/D
All ASA attendees welcome to attend
Program Co-Chairs:
Mahmound Aarabi, MD, PhD
Luke Simon, PhD
**Not CME Accredited*

4:30 p.m. – 4:35 p.m. **Opening Remarks**
Mahmound Aarabi, MD, PhD
Luke Simon, PhD

4:35 p.m. – 5:20 p.m. **Publish, Don't Perish Preparing a High-Impact Manuscript**
Moderator:
Mahmoud Aarabi, MD, PhD
Basic Research View:
Sarah Kimmins, PhD
Clinical Outlook:
Darius A Paduch, MD, PhD

5:20 p.m. – 5:35 p.m. **Break**

5:35 p.m. – 6:20 p.m. **A Prism Perspective of Scientific Review Addressing the Process at All Levels**
Moderator:
Luke Simon, PhD
Editor:
John K. Amory, MD, MPH
Reviewer:
Michael L. Eisenberg, MD
Authors:
Sarah Kimmins, PhD
Darius A. Paduch, MD, PhD

6:20 p.m. – 6:30 p.m. **The Second Line Parade**
Sophie La Salle, PhD

6:30 p.m. – 8:30 p.m. **Trainee Forum and Mixer**
Location: Grand Gallery
(All Trainee Travel and Onsite Poster Awards (Outstanding Trainee Investigator, Anna Steinberger Award, and Trainee Merrit Awards) will be distributed and celebrated at this event)

MONDAY, APRIL 4, 2016

7:00 a.m. – 6:00 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

7:00 a.m. – 3:30 p.m. **Exhibit Hall Open**
Location: Astor Ballroom

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

7:00 a.m. – 8:00 a.m. **Industry Satellite Symposium Breakfast**
Location: Toulouse B (2nd Floor Mezzanine)

8:00 a.m. – 9:00 a.m. **WOMEN IN ANDROLOGY LECTURE**
Exploring the Genetic and Genomic Basis of Male Reproductive Defects
(Introduction: Sarah Kimmins, PhD)
Dolores J. Lamb, PhD, HCLD
Baylor College of Medicine

9:00 a.m. – 9:15 a.m. ***Matthew P. Hardy Young Andrologist Award**
(Introduced by Moira K. O'Bryan, PhD)
2016 Recipient: Lee B. Smith, PhD
**Not CME Accredited*

9:15 a.m. - 10:15 a.m. **Symposium II/ Debate**
Moderator: Kirk Lo, MD, FRCSC

Fertility Preservation in Adolescent Klinefelter Patients: the Case (or not) for Early Intervention
Darius A. Paduch, MD, PhD
Weill Medical College of Cornell University

Nathalie Rives
CHU Hôpitaux de Rouen - CHU Charles Nicolle, France

10:15 a.m. - 10:30 a.m. **Break**

10:30 a.m. - 11:15 a.m. **DIVERSITY LECTURE**
My Search for the Reasons for Health Disparities: What I Have Learned and What I Haven't
(Introduced by: Maria Christina W. Avellar, PhD)
Thomas Laveist, PhD
George Washington University

11:15 a.m. - 12:30 p.m. ***Poster Session II**
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

12:30 p.m. - 1:45 p.m. **Lunch On Own**

12:30 p.m. - 1:45 p.m. **WOMEN IN ANDROLOGY LUNCHEON**
Engaging Basic Scientists in Translational Research
Location: Toulouse B (2nd Floor Mezzanine)
Dolores J. Lamb, PhD, HCLD
Baylor College of Medicine

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SCHEDULE OF EVENTS

12:30 p.m. – 1:45 p.m. **Editorial Board Luncheon**
Location: Toulouse A (2nd Floor Mezzanine)

1:45 p.m. - 3:15 p.m. **SYMPOSIUM III- Novel Therapies in Andrology**
Moderator: John K. Amory, MD, MPH

Use of an Antimicrobial Peptide, LL 37, as a Vaginal Contraceptive
Nongnuj Tanphaichitr, PhD
University of Ottawa

Ghrelin Hormone in Wound Healing and Prevention of Scarring in the Post-operative Setting of Abdominal and Testicular Surgery
Kathleen Hwang, MD
The Warren Alpert Medical School of Brown University

Therapeutic Potential of Transplanted Immune Privilege Sertoli Cells: Unexpected Revelations about Sertoli Cell Dogmas
Jannette Dufour, PhD
Texas Tech University Health Sciences Center

3:15 p.m. - 3:30 p.m. **Break**

3:30 p.m. - 4:15 p.m. **LECTURE II**
(Introduced by Janice Bailey, PhD)
Environmental Programming of the Epigenome in Paternal Reproductive Health
Sarah Kimmins, PhD
McGill University

4:15 p.m. - 5:00 p.m. **LECTURE III**
(Introduced by Larry Lipshultz, MD)
The Role of the Urologist in the Era of IVF/ICSI
Alexander W. Pastuszak, MD, PhD
Baylor College of Medicine

5:00 p.m. - 6:00 p.m. **ASA Business Meeting**

7:00 p.m. – 10:00 p.m. ***Annual Banquet**
Location: Paddlewheeler Creole Queen
1 Poydras Street
New Orleans, LA 70130
Located in Spanish Plaza behind Hilton Riverside and next to Outlet Collection at the Riverwalk

7:00 p.m. - 8:00 p.m. Boarding
8:00 p.m. - 10:00 p.m. Cruising
(Boat will leave dock and return)

Shuttle bus to boat will leave at 6:45 p.m. from the Astor Crowne Plaza Canal Street Entrance and return after the event at 10:00 p.m.

TUESDAY, APRIL 5, 2016

7:00 a.m. – 8:00 a.m. **2017 Program Committee Meeting**
Location: St. Ann (2nd Floor Mezzanine)

7:00 a.m. – 12:30 p.m. **Registration/Information Desk Open**
Location: Second Floor Foyer

7:00 a.m. – 8:00 a.m. **Continental Breakfast**
Location: Grand Gallery

8:00 a.m. - 9:30 a.m. **SYMPOSIUM IV - Germ Cell/ Spermatogonial Stem Cell Biology**
Moderator: Kyle Orwig, PhD

Defining the Mechanisms Regulation Spermatogonial Differentiation
Chris Geyer, PhD
Brody School of Medicine at ECU

Promoting Recombination in Mouse Meiosis
Sophie La Salle, PhD
Midwestern University

Preservation of Fertility through Transplantation of Germ Cells and Grafting of Testicular Tissue
Ali Honaramooz, DVM, PhD
University of Saskatchewan

9:30 a.m. - 9:45 a.m. **Break**

9:45 a.m. - 10:45 a.m. **INTERNATIONAL LECTURE**
Clinical Markers of Gonadal Function in Prepuberty and Puberty
(Introduced by Patricia Cuasnicu, PhD)
Rodolfo A. Rey, MD, PhD
Hospital de Niños Ricardo Gutiérrez, Buenos Aires

10:45 a.m. - 12:15 p.m. **SYMPOSIUM V - Male Reproductive Tract/Sperm Biology**
Moderator: Sylvie Breton, PhD

Exploring the Role of Macrophages and Dendritic Cells in the Epididymis
Nicolas Da Silva, PhD
Massachusetts General Hospital

Y Chromosome and Spermatogenesis
Monika A. Ward, MSc, PhD
University of Hawaii

"Breaking" News from Spermatids
Guylain Boissonneault, PhD
Universite de Sherbrooke, Canada

MEETING ADJOURNED

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SCHEDULE OF EVENTS

***ASA Basic Science Workshop:**
"ANDRomics: Genomics, Epigenomics, and Proteomics of Male Fertility"
Saturday, April 2, 2016
8:30 a.m. – 4:30 p.m.

Program Chair: Cristian O'Flaherty, DVM, PhD
Location: Grand Ballroom A/B
**Not CME accredited*

8:30 a.m. - 8:35 a.m. **Welcome and Introduction**
Cristian O'Flaherty, DVM, PhD
McGill University -Research Institute-MUHC

8:35 a.m. - 10:05 a.m. **Session I: Proteomics and DNA Integrity Strategies**

8:35 a.m.-9:05 a.m. **Sperm Biomarkers for Male Infertility**
Mark Baker, PhD
University of Newcastle

9:05 a.m.-9:35 a.m. **The Study of Ion Channels in Sperm**
Celia Santi, PhD
Washington University

9:35 a.m.-10:05 a.m. **Assessment of Sperm Proteins and DNA Integrity by Flow Cytometry**
Elisabetta Baldi, PhD
Florence University

10:05 a.m. - 10:25 a.m. **Coffee Break**

10:25 a.m. - 12:05 p.m. **Selected Abstracts**

Mouse Models Incorporating Fluorescent Proteins to Study Signaling Pathways in Sertoli Cells *In Vitro* and *In Vivo*
Thomas Garcia, PhD

3-Dimensional Testicular Organoid: A Novel Tool For The Study Of Human Spermatogenesis And Gonadotoxicity In Vitro
Hooman Sadri-Ardekani, MD, PhD

Paternal Stress Reprograms Epigenetic Marks in Sperm And Epididymis: Implications For Long−Term Transmission Of Experience
Jennifer Chan, PhD Candidate; Tracy Bale, PhD

Leveraging Available Molecular Genetic Tools to Investigate the Function of Genes Important for Sperm Function and Fertility Using *Caenorhabditis Elegans*
Matthew Marcello, MD

Genomic Studies in Azoospermic and Oligozoospermic Males with Male Infertility
Alexander Yatsenko, MD

12:05 p.m. - 1:00 p.m. **Lunch with Keynote Speaker: Functional Genomic Approaches to Define the Stem Cell State in Male Germ Cells**
Jon M. Oatley, PhD
Washington State University

1:00 p.m. - 4:20 p.m. **Session II: Epigenetics, Genome-Scale Tools and Genetics Models**

1:00 p.m. - 1:20 p.m. **Assessing the Sperm DNA Methylome – Past and Future**
Jacquetta Trasler, PhD
McGill University

1:20 p.m. - 1:50 p.m. **Whole Genome Sequencing-Based Approaches to Assess the Sperm DNA Methylome**
Mahamoud Aarabi, MD, PhD
McGill University

1:50 p.m. - 2:20 p.m. **Targeted and Regional Assessment of the Sperm Epigenome**
Timothy Jenkins, PhD
University of Utah

2:20 p.m. - 2:50 p.m. **Coffee Break**

2:50 p.m. - 3:20 p.m. **Genetic Models in Reproductive Studies**
Sophie La Salle, PhD
Midwestern University

3:20 p.m. - 3:50 p.m. **Changing Gene Expression in Isolated Gonocytes**
Martine Culty, PhD
McGill University, Research Institute MUHC

3:50 p.m. – 4:20 p.m. **Reveal the Physiological Function of miRNAs in Vivo**
Wei Yan, PhD
University of Nevada

4:20 p.m. - 4:30p.m. **Closing Remarks**
Elizabeth Snyder, PhD
BW Chair, Jackson Laboratory

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SCHEDULE OF EVENTS

***ASA Andrology Lab Workshop:**
"Restoring Strict Morphology Relevance:
A Consensus Workshop"
Saturday, April 2, 2016
8:30 a.m. – 5:30 p.m.

Location: Astor Ballroom III

*The ALW has been approved by the **American Board of Bioanalysis (ABB)** Professional Enrichment Educational Renewal (PEER) for **0.85** CEU's or **8.5** contact hours of continuing education activity.*

8:30 a.m. - 8:35 a.m.	**Welcome from ALW Committee** Erma Z. Drobnis, PhD *University of Missouri School of Medicine*
8:35 a.m. - 8:45 a.m.	**Purpose of the Workshop and Ground Rules** Steven M. Schrader, PhD *CDC/NIOSH*
8:45 a.m. - 9:15 a.m.	**Classification I:** Participants
9:15 a.m. - 9:45 a.m.	**Classification II:** Participants
9:45 a.m. - 10:15 a.m.	**Classification III:** Participants
10:15 a.m. - 10:30 a.m.	**Break**
10:30 a.m. - 10:50 a.m.	**WHO's on Fifth: How Morphology Lost Predictive Value** Susan A. Rothmann, PhD, HCLD *Fertility Solutions, Inc.*
10:50 a.m. -11:10 a.m.	**Results of Classification Sessions** Steven M. Schrader, PhD *CDC/NIOSH*
11:10 a.m. - 11:30 a.m.	**Algorithm Rationale** Susan A. Rothmann, PhD, HCLD *Fertility Solutions, Inc.*
11:30 a.m. - 12:00 p.m.	**Algorithm Mechanics** Anna-Marie Bort, MLT(ASCP)CME *Fertility Solutions, Inc.*
12:00 p.m. - 1:30 p.m.	**LAB SCIENCE FORUM LUNCHEON** **Using Immunodetection Techniques for Assessing Fertility** Marie-Claude Hofmann, PhD *University of Texas - MD Anderson Cancer Center*
1:30 p.m. - 2:00 p.m.	**Algorithm Worked Examples** Anna-Marie Bort, MLT(ASCP)CME *Fertility Solutions, Inc.*
2:00 p.m. - 2:40 p.m.	**Classification I**: Participants
2:40 p.m. - 3:20 p.m.	**Classification II:** Participants
3:20 p.m. - 3:30 p.m.	**Break**
3:30 p.m. - 4:10 p.m.	**Classification III:** Participants
4:10 p.m. - 5:30 p.m.	**Results of Classification** Steven M. Schrader, PhD *CDC/NIOSH* and **Discussion of Controversial Classifications by Faculty**
5:30 p.m.	**Adjourn**

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SCHEDULE OF EVENTS

ASA Special Symposium
"What's New in Men's Health"
Saturday, April 2, 2016
1:00 p.m. - 5:15 p.m.

Program Chairs:
Mohit Khera, MD and Tobias S. Kohler, MD, MPH, FACS
Location: Grand Ballroom C/D

1:00 p.m. – 2:15 p.m. **Prostate Health**

1:00 p.m. - 1:30 p.m. **New Treatment Paradigms**
Charles Welliver, Jr., MD
Albany Medical Center

1:30 p.m. - 2:00 p.m. **Testosterone is Bad, No Wait Good For the Prostate**
Michael J. Butcher, DO
Park Nicollet Urology

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

2:15 p.m. - 2:30 p.m. **Break**

2:30 p.m. – 3:45 p.m. **Genital Health**

2:30 p.m. - 3:00 p.m. **Peyronie's Disease – What's New**
Eric L. Labord, MD
Ochsner Medical Center

3:00 p.m. - 3:30 p.m. **Varicocele Repair in the Era of IVF/ ICSI**
Jared Moss, MD
Northwestern University

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

3:45 p.m. - 4:00 p.m. **Break**

4:00 p.m. - 5:15 p.m. **Overall Men's Health**

4:00 p.m. - 4:30 p.m. **Obesity and Infertility**
Jared M. Bieniek, MD
Geisinger Medical Center

4:30 p.m. - 5:00 p.m. **Life Style Change and Sexual Function**
Abdulmaged M. Traish, PhD
Boston University - School of Medicine

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

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SPEAKER ABSTRACTS

SATURDAY, APRIL 2, 2016
6:20 p.m. - 7:30 p.m.

EMIL STEINBERGER MEMORIAL LECTURE
CATSPERS: SPERM FLAGELLA-SPECIFIC ION CHANNELS REQUIRED FOR MALE FERTILITY
David E. Clapham, MD, PhD
HHMI, Boston Children's Hospital, Harvard Medical School

CatSper ion channels are calcium permeant ion channels in the flagellar membrane of spermatozoa and are required for mouse and human male fertility. The core genes encoding this channel originated in early uniflagellated organisms, but additional subunits have been incorporated into the complex over evolution to mammals. At least nine genes encode subunits of the mammalian CatSper channel complex. Here I describe their localization in the flagellum and their function in the sperm's ability to reach and fertilize the egg. The CatSper channels are ideal targets for contraception, but may also be targeted for some forms of male infertility.

SUNDAY, APRIL 3, 2016
8:00 a.m. – 9:00 a.m.

AUA LECTURE
THE CHANGING PARADIGM OF PROSTATE CANCER TREATMENT: GENOMICS AND BEYOND
Kenneth J. Pienta, MD
Johns Hopkins University

The last 24 months has seen paradigm shifts in our understanding of prostate tumorigenesis with significant implications for therapy. Recent studies, striving to define precision medicine in men newly diagnosed as well as metastatic castrate resistant prostate cancer have demonstrated that unique molecular alterations can be utilized for prognostic as well as therapeutic decisions. For example, whole-exome and transcriptome sequencing of men with mCRPC revealed frequent aberrations of AR, ETS genes, TP53, and PTEN (40%-60% of cases). Genomic aberrations in BRCA2, BRCA1, and ATM have been observed at substantially higher frequencies (19.3% overall) in mCRPC compared to those in primary prostate cancers. Identified aberrations in these DNA repair pathway genes are associated with a high response rate to platinum chemotherapy as well as PARP inhibitors. Furthermore, alternate splicing in patients with mCRPC being treated with supra-castration agents can lead to expression of variants of the androgen receptor, which are associated with poor outcomes.
It is clear, however, that understanding the genomic landscape of cancers is rarely enough to cure patients with metastatic disease. As they grow, tumors fundamentally alter their microenvironment, disrupting the homeostasis of the host organ and eventually the patient as a whole. Lethality is the ultimate result of deregulated cell signaling and regulatory mechanisms as well as inappropriate host cell recruitment to the sites of tumor. These processes are best understood within the framework of ecological biology: multiple interacting ecosystems (organ systems) within a larger biosphere (body), alterations in species stoichiometry (host cell types), resource cycling (cellular metabolism and cell-cell signaling) and ecosystem collapse (organ failure and death). In particular, as cancer cells generate their own niche within the tumor ecosystem, ecological engineering and autoeutrophication displace normal cell function and result in the creation of a hypoxic, acidic and nutrient-poor environment. This "cancer swamp" has genetic and epigenetic effects at the local ecosystem level to promote metastasis and at the systemic host level to induce cytokine-mediated lethal syndromes, a major cause of death of cancer patients.

SUNDAY, APRIL 3, 2016
9:15 a.m. – 9:45 a.m.

SYMPOSIUM I – Somatic Cells of the Testis
PUTTING THE BRAKES ON STEROIDOGENESIS: NOVEL INSIGHTS INTO THE FINE REGULATION OF STEROID HORMONE PRODUCTION
Jacques J. Tremblay[1,2], Francis Bergeron[1], Houssein S. Abdou[1]
[1]Reproduction, Mother and Child Health, Centre de recherche du CHU de Québec-Université Laval, Québec, Canada
[2]Centre for Research in Reproduction, Development and Intergenerational Health, Department of Obstetrics, Gynecology and Reproduction, Faculty of Medicine, Université Laval, Québec, Canada

Steroid hormones regulate essential physiological processes and inadequate levels are associated with various pathological conditions. Consequently, the process of steroid hormone biosynthesis must be finely regulated. In testosterone-producing Leydig cells, steroidogenesis is strongly stimulated by LH via its receptor located on the surface of Leydig cells. This stimulates adenylate cyclase leading to increased cAMP production and activation of signaling pathways and kinases. Ultimately, these kinases phosphorylate target proteins, including transcription factors leading to increased expression of genes involved in steroid hormone synthesis such as the steroidogenic acute regulatory protein STAR, which is essential for the initiation of steroidogenesis. So far, PKA was believed to be the main kinase activated by cAMP. However, other kinases are also involved including MAPK and CAMKI. We found that CAMKI is activated downstream of cAMP in Leydig cells and is essential for hormone-induced steroidogenesis. In the classical negative feedback loop model, steroid hormones will then feedback on the hypothalamus/pituitary to repress LH production. Without LH, steroidogenesis passively decreases as cAMP is degraded into AMP by phosphodiesterases. Our lab reported another cell autonomous mechanism that actively represses steroidogenesis both in Leydig and adrenal cells. We reported that the increase in intracellular AMP levels following cAMP degradation leads to activation of AMPK. Activated AMPK then actively inhibits hormone-induced steroidogenesis by repressing the expression of key regulators of steroidogenesis including STAR. Activation of the repressive pathway (AMPK) occurs after activation of the stimulatory (CAMKI) pathway. This two-step mechanism first stimulates steroid hormone production before repressing it to prevent overproduction. This ensures that accurate steroid hormone levels are produced. An overview of the current state of knowledge as well as potential molecular mechanisms of action of CAMKI and AMPK will be presented.
Funding: Supported by the Canadian Institutes of Health Research.

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SPEAKER ABSTRACTS

SUNDAY, APRIL 3, 2016
9:45 a.m. – 10:15 a.m.

SYMPOSIUM I – Somatic Cells Of The Testis
MACROPHAGES: DIVERSE PLAYERS IN TESTIS BIOLOGY
Tony DeFalco, PhD
Cincinnati Children's Hospital Medical Center

Introduction: Organ formation and homeostasis rely on extensive interactions between diverse cell types. In these processes, the functions of immune cells such as macrophages are under-appreciated, despite their virtually ubiquitous presence in fetal and adult organs. Macrophages are traditionally known for their roles in innate immunity, but recent evidence indicates that they play diverse and vital functions in development and health. However, the role of macrophages in fetal testis organogenesis and adult spermatogenesis are poorly understood.
Methods: We used confocal microscopy and flow cytometry to identify macrophages in early stages of testis morphogenesis and to characterize macrophages in the adult testis of mice. We specifically ablated macrophages using Cre-lox-mediated diphtheria toxin methods (*Rosa*-DTA or *Rosa*-DTR) in fetuses or transiently in adults. We examined the effects of macrophage ablation on fetal testis morphogenesis (e.g., cord formation and vascular remodeling) and adult spermatogenesis (e.g., spermatogonial differentiation).
Results: We observed that macrophage-depleted fetal testes showed reduced vascular remodeling and disrupted testis cord formation. Transient ablation of macrophages in adult testes resulted in defects in spermatogonial differentiation, likely independent of previously identified roles for macrophages in steroidogenesis.
Conclusions: Our results suggest that macrophages are required for vascular remodeling and tissue remodeling in fetal stages of testis morphogenesis, as well as for promoting spermatogenesis in the adult testis, likely via secreted factors that drive spermatogonial stem cell differentiation. Our findings demonstrate that macrophages are an unexpectedly critical cell type in testicular organogenesis and homeostasis.
Funding: March of Dimes Foundation (Basil O'Connor Starter Scholar Research Award); Cincinnati Children's Hospital Medical Center (CCHMC) Trustee Grant Award; CCHMC Research Innovation and Pilot Funding; and CCHMC developmental funds.

SUNDAY, APRIL 3, 2016
10:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Somatic Cells of the Testis
REVISITING THE CONCEPT OF TERMINAL DIFFERENTIATION IN ADULT SERTOLI CELLS
Peter Stanton, PhD
Hudson Institute of Medical Research, and Monash University, Australia

Introduction: In mammals, Sertoli cells proliferate during foetal and early neonatal life, then at puberty undergo a series of differentiation steps to yield the mature, non-proliferative 'adult' cell capable of supporting spermatogenesis. This process has been commonly thought to be non-reversible, thereby establishing the concept that adult Sertoli cells are terminally differentiated. Mounting evidence from our lab, and others, now suggests that functionally mature Sertoli cells retain the ability to become proliferative and also display other characteristics of the immature population, including protein marker expression and loss of tight junctions. Recently, we characterised activin A, a member of the TGFβ superfamily, as the first growth factor able to drive Sertoli cells from a mature to an immature state.
Methods: An AAV6-activin A viral construct administered to the adult mouse tibialis anterior leg muscle for four weeks was used to elevate circulating activin A *in vivo*. Additionally, an *in vitro* post-pubertal (20d) rat primary Sertoli cell system was employed with endpoints including proliferation, tight junction function and protein marker expression.
Results: *In vivo*, a 4-fold increase in serum activin A caused a 25% reduction in testis weights with a heterogeneous testicular phenotype. This included Sertoli cell-only tubules with loss of tight junction (TJ) proteins and disrupted blood-testis barrier (BTB) function, whilst other tubules retained BTB function but displayed a loss of specific germ cell types (spermatogonia, elongated spermatids). *In vitro*, activin A dose-dependently caused an almost total loss of TJ function and TJ protein localisation, a 5-fold increase in cell proliferation and expression of cell-cycle genes, and a dramatic increase in cytokeratin-18 expression, which is a marker of immature Sertoli cells.
Conclusions: Differentiated Sertoli cells retain the ability to de-differentiate into a functionally immature state. These models provide a means to dissect the pro- and anti- proliferative signalling pathways that operate on Sertoli cells in the adult seminiferous epithelium and suggest that de-differentiation could be an additional mechanism leading to spermatogenic failure.

SUNDAY, APRIL 3, 2016
3:45 p.m. – 4:30 p.m.

LECTURE I
STEROID-BINDING PROTEINS- GATEKEEPERS OF ANDROGEN ACTION
Geoffrey L. Hammond, PhD
Departments of Cellular & Physiological Sciences and Obstetrics and Gynaecology, The University of British Columbia, Vancouver, BC V6T 1Z3, Canada.

Biologically active androgens (testosterone and 5α-dihydrotestosterone) are transported in the blood by albumin and sex hormone-binding globulin (SHBG), which together regulate their non-protein bound or "free" fractions that are considered to be biologically active. As such these plasma proteins can be viewed as the "primary gatekeepers" of androgen action.
Albumin binds steroids with limited specificity and low affinity but its high concentration in blood buffers major fluctuations in androgen concentrations and their free fractions. By contrast, SHBG is the key determinant of plasma testosterone levels, and it plays a more dynamic role in controlling steroid bioavailability. Human SHBG binds androgens with nM affinity but also binds estradiol. These sex steroids interact with a single SHBG binding site differently, and this may influence how SHBG binds to other proteins in extravascular compartments of some tissues. Human SHBG also binds calcium and zinc, which are integral to its functions, and its glycosylation state influences its plasma clearance.
As in other mammals, the single *SHBG* gene in humans is expressed in the testis. While expression of *Shbg* in Sertoli cells in many other mammals produces the SHBG homolog, known as the

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androgen-binding protein, human *SHBG* expression in the testis is confined to germ cells, and produces an SHBG isoform that accumulates in the acrosome. The function of this SHBG isoform remains unknown, but its levels in the acrosome decline with age and correlate with sperm motility. Evidence from "humanized" transgenic mice indicates that *SHBG* expression in some epithelial cells also produces an SHBG isoform that remains within the cytoplasm and influences androgen access to the androgen receptor.

Hepatic SHBG production varies during development and different physiological and pathophysiological conditions, and abnormalities in its plasma levels or its ability to bind steroids are associated with a variety of pathologies. Understanding how the structure of SHBG determines its specialized functions, how *SHBG* expression is controlled, and how SHBG functions both within and outside the blood provides insight into how androgens act in health and disease.

MONDAY, APRIL 4, 2016
8:00 a.m. – 9:00 a.m.

WOMEN IN ANDROLOGY LECTURE

EXPLORING THE GENETIC AND GENOMIC BASIS OF MALE REPRODUCTIVE DEFECTS

Dolores J. Lamb, PhD
Center for Reproductive Medicine, Scott Department of Urology, and the Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

Male reproductive defects encompass a wide range of developmental and adult phenotypes and are among the most common birth defects with non-obstructive azoospermia being present in about 4% of men, hypospadias being present in about 1 in 250, and cryptorchidism being present in 1 in 30 full-term births. Even ambiguous genitalia is relatively common, occurring in about 1 in 4000 births. For patients with male reproductive defects and their families, these defects have significant consequences, including the need for surgical correction (sometimes with future surgical complications), infertility requiring the use of surgical intervention, the use of assisted reproductive technologies to attempt to achieve a pregnancy, and, for some patients, sterility affecting their entire life-plan. In recent years there is a growing awareness that genetic and genomic defects can impact reproductive health throughout the lifespan. Karyotypes are used to identify numerical and structural abnormalities associated with birth defects in general, but more recently "molecular karyotypes" or array comparative genomic hybridization (aCGH) have been developed to identify much smaller but equally potent disease-causing genomic aberrations. aCGH platforms now span the entire genome to identify microdeletions, small submicroscopic losses, or microduplications (gains) of chromosomal regions that may result in altered gene dosage affecting male reproductive development and/or function. Indeed, microdeletion/microduplicaton "hot-spots" have been identified on the Y chromosome (impacting spermatogenesis and stature), the X chromosome (cryptorchidism and hypospadias), and 9p24.3, 22q11 and 16p11.2 affecting both upper and lower genitourinary tract development and testicular function. Other microdeletions are known affect sperm morphology and fertilizing potential. Genomic alterations such as these microdeletions and microduplications described above underlie not only a variety of male reproductive defects, but are associated with a wide range of birth defects, autism spectrum disorders, and genomic diseases and syndromes.

This study was supported in part by US National Institutes of Health grant K12 DK0083014 (KURe,) R01DK078121 and 5T32DK007763 from the National Institute of Diabetes and Digestive and Kidney Diseases (to D.J.L.)

MONDAY, APRIL 4, 2016
9:15 a.m. – 10:15 a.m.

SYMPOSIUM II/DEBATE

FERTILITY PRESERVATION IN ADOLESCENT KLINEFELTER PATIENTS: THE CASE (OR NOT) FOR EARLY INTERVENTION

Nathalie Rives, MD, PhD[1] and Darius A. Paduch, MD, PhD[2]
[1]Rouen University Hospital, Normandy University and [2]Weill Medical College of Cornell University

Objectives: Klinefelter syndrome (KS) is the most common form of hypogonadism and chromosome aneuploidy that affects 1 in 500 males at birth. A wide variability in clinical expression has been observed in KS males, related to the timing and degree of androgen deficiency. Male hypogonadism observed in KS males is exceptionally suspected in pre-pubertal boys but remains detectable from mid- to late puberty when germ cell apoptosis, sclerosis and hyalinization of the seminiferous tubules affect drastically testis development, volume and function. Consequently, less than 10% of expected XXY subjects are diagnosed before the onset of puberty. Considering that germ cell depletion is markedly affected at the onset of puberty and continues throughout KS life, this observation raises the question of a precocious intervention in KS patients to be able to cryopreserve immature germ cells or spermatozoa.

Methods: Evidence from published medical literature, as well as findings from our research team, will be presented.

Results: Spermatozoa have occasionally been identified in ejaculate of adult KS but very exceptionally in KS adolescents. Spermatozoa can also be retrieved in testicular tissue of adolescent KS patients. Furthermore, testis may also arbor spermatogonia and non-completely differentiated germ cells. The physician should discuss with the patient and his parents over a several-month long period before performing semen sample or bilateral testicular biopsy. However, neither clinical features nor hormonal parameters could predict germ cell recovery in adult or adolescent KS patients. No predictive factors can actually demonstrate that early diagnosis of KS would allow increasing the chance of spermatozoa retrieval considering even if it is considered that semen quality of KS patients may decline with age. Leydig cell dysfunction may also be another factor that may affect spermatogenesis process in XXY adolescents. Fertility preservation might be preferentially proposed in adolescent Klinefelter patient when semen sample is possible but not yet after the onset of puberty and when the patient is able to consider alternative options to become father and to accept germ cell retrieval failure. However, precocious diagnosis of Klinefelter syndrome also has to be considered because that may not solely improve the possibility of fertility preservation after the onset of puberty but also the medical care and the quality of life of these patients.

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SPEAKER ABSTRACTS

MONDAY, APRIL 4, 2016
10:30 a.m. – 11:15 a.m.

DIVERSITY LECTURE
MY SEARCH FOR THE REASONS FOR HEALTH DISPARITIES: WHAT I HAVE LEARNED AND WHAT I HAVEN'T
Thomas A. LaVeist, PhD
Chair and Professor, Department of Health Policy and Management, Milken Institute School of Public Health, George Washington University

More than two decades ago, I learned that there were race disparities in health. Today that fact is common knowledge, but it was less common then. I wondered why these disparities existed and sought answers in the research literature. Surprised by the lack of satisfying answers, I devoted my career to finding one. In this lecture, I will describe my personal journey to discover the answer and describe a new direction to explore the hemisphere to see what lessons can be learned from other African descendants of the transatlantic slave trade.

MONDAY, APRIL 4, 2016
1:45 p.m. – 2:15 p.m.

SYMPOSIUM III – Novel Therapies in Andrology
USE OF AN ANTIMICROBIAL PEPTIDE, LL-37, AS A VAGINAL CONTRACEPTIVE
Nongnuj Tanphaichitr
Chronic Disease Program, Ottawa Hospital Research Institute; and Department of Obstetrics and Gynecology and Department of Biochemistry, Microbiology, Immunology, Faculty of Medicine, University of Ottawa; Ottawa, Ontario, CANADA

The continuing increases in global population and sexually transmitted infection (STI) demand a search for compounds with dual contraceptive and microbicidal activities. Attempts to develop surfactant nanoxynol-9, the only vaginal spermicide available over the counter, into a microbicide were not only unsuccessful, but also implicating a higher risk of STI after its vaginal use. Alternatively, antimicrobial peptides (AMPs) could be screened for spermicidal activity. AMPs are peptides of <10 kDa with amphipathic and cationic property. Secreted as part of innate immunity, AMPs have a wide spectrum of microbicidal activities-on Gram+/- bacteria, yeasts and viruses. Among 12 spermicidal AMPs described, LL-37 is the most promising to be developed further as a vaginal spermicide/microbicide. First, it is microbicidal to most STI microbes. LL-37 is also the only human spermicidal AMP, naturally produced in the vagina via processing from its precursor hCAP-18 (a seminal plasma component), 2-6 post-coitus. At this time motile sperm have already swum into the uterine cavity; hence, the LL-37 produced is rather for protection of the vaginal epithelium from microbial attacks. In fact, mouse/human sperm treated with LL-37 (3.6 and 10.8 μM, respectively) became immotile and prematurely acrosome reacted and lost their membrane intactness. This last effect of LL-37 on sperm is analogous to its action on bacterial membranes, which is initiated by interaction between cationic LL-37 and membrane anionic lipids (phosphatidylglycerol & cardiolipin). Sperm uniquely possess an anionic lipid, sulfogalactosylglycerolipid (SGG), for which LL-37 had direct affinity. Significantly, LL-37-treated mouse sperm lost the ability to fertilize eggs in vitro. Furthermore, female mice naturally cycling to the estrus day, which were transcervically injected with sperm with LL-37 failed to become pregnant (n=26). In contrast, pregnancies occurred in 24 of 26 females injected with sperm alone. No adverse effects were observed in the reproductive tract of females injected with LL-37. Our results therefore indicate a great potential in development of LL-37 or its truncated peptides into a vaginal contraceptive/microbicide.
Funding: Supported by CIHR and previously the Bill & Melinda Gates Foundation

MONDAY, APRIL 4, 2016
2:15 p.m. – 2:45 p.m.

SYMPOSIUM III – Novel Therapies in Andrology
GHRELIN HORMONE IN WOUND HEALING AND PREVENTION OF SCARRING IN THE POST-OPERATIVE SETTING OF ABDOMINAL AND TESTICULAR SURGERY
Kathleen Hwang, MD
The Warren Alpert Medical School of Brown University

Objective: Peritoneal adhesion formation is a well-recognized consequence of abdominal and pelvic surgery, causing infertility, chronic pelvic pain and intestinal obstruction. We hypothesized that ghrelin, a 28 amino acid peptide predominantly found in the stomach, plays an important role in preventing post-operative surgical adhesions. We aimed to develop a new surgical peritoneal adhesion model to define the role that ghrelin plays in wound healing and adhesion formation, as well as start to define the molecular mechanisms behind these anti-adhesion effects.
Methods: Experimental post-surgical adhesions were created in C57BL/6 wild type mice is combination of ischemic peritoneal buttons and cecal multiple abrasions. Ghrelin or saline intraperitoneal injections were given twice daily from two days before surgery to selected time points post-surgically to assess the phenotypic and molecular effects of treatment (1 day (n=20), 4 days (n=20), and 20 days (n=40) after surgery). Endpoints included the scoring of adhesions and gene and protein expression analysis of pro-fibrogenic factors conducted on peritoneal ischemic tissue by quantitative PCR and Western blot.
Results: The novel mouse model demonstrated consistent and easily reproducible formation of intra-abdominal adhesions and down-regulated pro-inflammatory gene and protein expression, including Tgfb3 and Tgfbr2. Ghrelin administration significantly reduced post-operative adhesion formation (p-value < 0.001) in wild type mice. The anti-adhesion effect of ghrelin seen in wild type mice was not detected in GHSR KO mice demonstrating that this effect is mediated by the GHSR-1a receptor. The up-regulation of inhibitory proteins Smad6 and Smad7 confirmed the ghrelin-induced blockage of TGF-β/Smad signaling. Further, ghrelin exposure significantly decreases gene expression of Il4, Il13ra2 and Tnf, up-stream factors involved in the activation of TGF-β signaling pathways.
Conclusions: Ghrelin is a candidate therapeutic drug for post-operative adhesion prevention and many surgical fields, inhibiting inflammatory responses via blockage of the TGF-β/Smad signaling pathway.

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SPEAKER ABSTRACTS

MONDAY, APRIL 4, 2016
2:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Novel Therapies in Andrology

THERAPEUTIC POTENTIAL OF TRANSPLANTED IMMUNE PRIVILEGE SERTOLI CELLS: UNEXPECTED REVELATIONS ABOUT SERTOLI CELL DOGMAS

Gurvinder Kaur, PhD, Lea Ann Thompson, MS, Rachel Dziuk and Jannette M. Dufour, PhD
Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas USA

The testis has long been recognized as an immune privilege site. Sertoli cells (SC) are considered key players in creating this immune privileged environment, which has led to their use in transplantation. For instance, SC protect co-grafted cells when transplanted as allografts or xenografts. Contrary to popular belief, this protection involves more than sequestering immunogenic cells behind the blood testis barrier and also includes production of immunomodulatory factors. Based on their unique survival after transplantation, we hypothesized SC could be engineered to express basal levels of insulin. SC transduced with a recombinant adenoviral vector containing furin-modified human proinsulin cDNA expressed insulin mRNA and protein. Moreover, transplantation of 5-20 million insulin-expressing SC into diabetic SCID mice, significantly lowered blood glucose levels, demonstrating the ability of SC to produce functional insulin. However, the lowering of blood glucose and expression of insulin were transient, which could be due to the epichromosomal nature of the adenoviral vector combined with proliferation of prepubertal SC. Therefore, SC isolated from "terminally differentiated" nondividing, postpubertal rats were transduced with the same adenoviral vector and transplanted into diabetic SCID mice. However, the expression of insulin and decrease in blood glucose was still transient. Surprisingly, immunohistochemical analysis of the grafts for BrdU and MKI67 indicated that nondividing SC resumed proliferation after transplantation, which is contrary to previous studies demonstrating that SC from postpubertal rodents are terminally differentiated. Next, SC were transduced with a lentiviral vector (for stable expression) containing furin-modified human proinsulin cDNA. The transduced SC expressed insulin mRNA and protein for more than two years (in vitro). Moreover, insulin-expressing SC survived and expressed insulin for over 50 days when transplanted as allografts in diabetic BALB/c mice. Overall, our data demonstrate that SC can stably produce insulin and retained their immune privileged properties thereby supporting the use of SC in cell based gene therapy.

Funding: Supported by NICHD, NIAID and Wilson Foundation.

MONDAY, APRIL 4, 2016
3:30 p.m. – 4:15 p.m.

LECTURE II

ENVIRONMENTAL PROGRAMMING OF THE EPIGENOME IN PATERNAL REPRODUCTIVE HEALTH

Sarah Kimmins, Romain Lambrot, Keith Siklenka and Christine Lafleur
Departments of Animal Science and Pharmacology and Therapeutics, McGill University, Montreal Quebec, Canada

Despite the father transmitting half the heritable information to the embryo, the focus on preconception health has largely been on the mother. New studies highlight the role of the father in disease transmission via non-genetic inheritance, through epigenetic mechanisms. Epigenetic mechanisms include, DNA methylation, post-translational modifications of histones and noncoding RNAs. Paternal effects have been linked to developmental abnormalities and complex diseases such as cancer, diabetes and obesity. Studies in humans and animals have linked epigenetic inheritance to the transmission of environmentally induced phenotypic traits from the father to the developing embryo, and these have been associated with altered gene expression and developmental abnormalities in first and second offspring generations. The mechanisms underlying such transmission remain unresolved. The genome in spermatozoa is packaged in a highly unique manner where the majority is packaged by sperm-specific nucleoproteins, the protamines. Intriguingly, there are regulatory regions of many genes that retain histones and these carry posttranslational modifications. If histone methylation is altered in sperm development, there are dire consequence for embryo development that is transgenerational (Siklenka et al., 2015; *Science*). In mice, environmental exposures such as diet and toxicants alter the sperm epigenome, but whether there are similar responses in men is unknown. Our overall objectives are to: 1) determine when histone methylation patterns that are transmitted to the embryo are established in spermatogenesis, and 2) whether environmental exposures can alter sperm histone methylation patterns. Using epigenomic approaches, such as chromatin immunoprecipitation followed by sequencing (ChIP-seq), we have been characterizing the effects of environmental exposures on spermatogonia and sperm from mice and men. Our long-term goals are to identify windows in spermatogenesis that are sensitive to reprogramming and to determine the potential for interventions to correct environment induced epimutations.

Funding: This research has been funded by the Canadian Institutes of Health Research.

MONDAY, APRIL 4, 2016
4:15 p.m. – 5:00 p.m.

LECTURE III

THE ROLE OF THE UROLOGIST IN THE ERA OF IVF/ICSI

Alexander W. Pastuszak, MD, PhD

The advent of *in vitro* fertilization (IVF), specifically the introduction of intracytoplasmic sperm injection (ICSI) in 1993, revolutionized assisted reproductive technology (ART) and the treatment of male infertility. For the first time, the union of single sperm with eggs could provide the possibility of parenthood for otherwise hopelessly infertile couples. Over the past two decades, the popularity and use of IVF/ICSI has dramatically increased, to the point where it is the preferred ART. As part of this trend, however, male patients are less frequently referred to and evaluated by urologists, despite growing evidence that male infertility confers risks associated with general men's health. Male infertility is associated with an increased incidence of numerous cancers, hypogonadism, and other general medical conditions, as well as an increased mortality risk. Rather than serving as the gateway to evaluation and long-term treatment of these men, urologists are serving as sperm retrieval experts, and these male patients are left without the care needed to properly address the negative sequelae of infertility. Such an approach goes against established guidelines for the treatment of the infertile couple, which recommend both male and female partner evaluations. In this talk, we will discuss

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the role of the urologist in the era of IVF/ICSI, with the emphasis on the pivotal role that the urologist should play in the evaluation and treatment of the infertile male. The urologist is essential in diagnosing serious or life-threatening conditions associated with infertility, treating the reversible causes of male infertility, defining the untreatable causes of testis failure, and lastly, facilitating sperm retrieval.

TUESDAY, APRIL 5, 2016
8:00 a.m. – 8:30 a.m.

SYMPOSIUM IV - Germ Cell/Spermatogonial Stem Cell Biology
"DEFINING THE MECHANISMS REGULATING SPERMATOGONIAL DIFFERENTIATION"
Nicholas D. Serra[1], Ellen K. Velte[1], Bryan A. Niedenberger[1], Randall H. Renegar[1], and Christopher B. Geyer[1,2]
[1]Department of Anatomy and Cell Biology, Brody School of Medicine, [2]East Carolina Diabetes and Obesity Institute at East Carolina University, Greenville, NC

Objectives: Spermatogonial stem cells (SSCs) must balance self-renewal with production of transit-amplifying progenitors that differentiate in response to retinoic acid (RA) before entering meiosis. This self-renewal vs. differentiation fate decision is critical for maintaining tissue homeostasis, as imbalances cause defects that can lead to human testicular cancer or infertility. Little is currently known about the program of differentiation initiated by RA, and the pathways and proteins involved are poorly defined. We recently found that RA stimulation of the PI3K/AKT/mTOR kinase signaling pathway is required for differentiation. Short-term inhibition of mTOR complex 1 (mTORC1) by rapamycin blocked spermatogonial differentiation in vivo and prevented RA-induced translational activation.
Methods: For the studies described below, we used two approaches to further assess mTORC1's specific role in spermatogenesis. First, we generated mTORC1 germ cell knockout mice to test the cell-autonomous requirement for this complex in developing spermatogonia. Second, we utilized a mouse model to compare the effects of rapamycin with those of its closely related analog Everolimus on spermatogenesis and male fertility. This is of particular relevance for human health, as Everolimus is an FDA-approved drug used in prevention of organ transplant rejection and in cancer chemotherapy.
Results: Reproductive phenotype of mTOR germ cell KO mice closely resembled that of rapamycin-treated mice, revealing a cell-autonomous requirement for mTORC1 activation in spermatogonial development. Similar to rapamycin, we found that Everolimus treatment from postnatal (P)20-50 dramatically reduced body and testis weights and epididymal sperm counts. Histological analysis revealed a general disorganization of the seminiferous epithelium with an apparent loss of differentiating spermatogonia. Importantly, decreased testis weights returned to normal after a one-month recovery period (P50-P120). However, sperm counts remained lower (~50% of controls) in both treatment groups at P120.
Conclusion: Our findings reveal that mTORC1 signaling is essential for spermatogonial proliferation and differentiation.
Funding: Funding provided by a grant from the NIH/NICHD (HD072552) to C.B.G.

TUESDAY, APRil 5, 2016
8:30 a.m. – 9:00 a.m.

SYMPOSIUM IV – Germ Cell/Spermatogonial Stem Cell Biology
PROMOTING RECOMBINATION IN MOUSE MEIOSIS
Sophie La Salle, PhD
Department of Biochemistry, Midwestern University, Downers Grove, Illinois

Introduction: Meiosis is a specialized type of cell division that allows for production of haploid gametes for sexual reproduction. Meiotic recombination ensures that chromosomes are physically linked through the exchange of DNA segments between homologs. In addition to creating genetic diversity, correct execution of meiosis prevents gamete aneuploidy and ensures fertility. Identifying the genetic program governing progression of germ cells through meiosis is therefore critical to understanding how healthy gametes are formed. We previously characterized the mouse ENU-induced *repro42* mutation which results in a nonsense codon in *spermatogenesis associated 22* (*Spata22*). Mutant *repro42* males and females are infertile due to meiotic arrest at the late zygotene stage, suggesting that SPATA22 plays an essential function during prophase I of meiosis. However, its precise contribution remains unclear.
Methods: We confirmed the requirement for *Spata22* by assessing a novel allele of this gene. Histological and cytological analyses were used to characterize mice carrying the targeted gene trap allele $Spata22^{Gt}$.
Results: Similar to *repro42* mutant mice, histological and mating analyses indicate that gametogenesis is profoundly affected in $Spata22^{Gt/Gt}$ males and females, resulting in infertility. Cytological examination confirms that germ cells do not progress beyond zygonema and meiotic arrest is linked to impairment of both synapsis and DNA repair. Analysis of SPATA22 distribution reveals that it localizes to foci associated with meiotic chromosomes during prophase I in both oocytes and spermatocytes. Furthermore, SPATA22 co-localizes with a number of proteins involved in meiotic recombination and is present until mid-pachynema, suggesting a role in resolution of recombination intermediates. Finally, analysis of $Spata22^{Gt/Gt}$ meiocytes uncovers that SPATA22 is required for localization of its interacting partner MEIOB.
Conclusion: Together, these results further support a role for SPATA22 during meiotic prophase and suggest that it is required for both meiotic recombination and synapsis in mice.
Funding: Supported by funds from Midwestern University.

TUESDAY, APRil 5, 2016
9:00 a.m. – 9:30 a.m.

SYMPOSIUM IV – Germ Cell/Spermatogonial Stem Cell Biology
Preservation of Fertility through Transplantation of Germ Cells and Grafting of Testicular Tissue
Ali Honaramooz, DVM, PhD
University of Saskatchewan

Not available at time of printing.

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SPEAKER ABSTRACTS

TUESDAY, APRIL 5, 2016
9:45a.m –10:45a.m.

INTERNATIONAL LECTURE
CLINICAL MARKERS OF GONADAL FUNCTION IN PREPUBERTY AND PUBERTY
Rodolfo A. Rey, MD, PhD
Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE), CONICET – FEI – División de Endocrinología, Hospital de Niños R. Gutiérrez, and Departamento de Histología, Biología Celular, Embriología y Genética, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

The hypothalamic-pituitary-testicular axis (HPT) is active during fetal life and the first three to six months after birth. Afterwards, during childhood, the axis is partially dormant: testosterone levels decrease following LH drop, and FSH secretion also decreases. Sertoli cells remain immature but active, as revealed by the high serum levels of anti-Müllerian hormone (AMH) and inhibin B. Therefore, testicular function before pubertal onset can be easily assessed by measuring Sertoli cell markers. Testicular AMH production increases in response to FSH and is potently inhibited by androgens. Inhibin B production is also stimulated by FSH. Hypogonadism can be evidenced, without the need for stimulation tests, by determining AMH and inhibin B. If Leydig cell function needs to be evaluated, testosterone and insulin-like factor 3 (INSL3) can be measured, but only after an hCG stimulation test. Serum AMH and inhibin B are undetectable in anorchid patients. Interestingly, gonadotropins may be within the normal range during childhood. In fetal-onset primary or central hypogonadism affecting the whole gonad, all testicular markers are low. Conversely, when hypogonadism only affects Leydig cells (e.g. LH/hCG insensitivity, sterodogenic enzyme defects), serum AMH and inhibin B are normal.
In normal and precocious puberty, the increase of intratesticular testosterone provokes an early decrease in serum AMH and an increase in serum inhibin B. Reversion to prepubertal levels are obtained after successful treatment with GnRH analogs or steroidogenic blocking agents. Serum AMH is a good marker to assess patient's compliance in the long term: while testosterone decreases in the days following a single dose, AMH only increases to prepubertal values if treatment is maintained for several months. In males of pubertal age with central hypogonadism, AMH and inhibin B are low. Treatment with FSH provokes an increase in serum levels of both Sertoli cell markers, whereas hCG administration increases testosterone levels. In patients with chromosome aneuploidies (Klinefelter or Down syndrome), Sertoli cell function is affected more readily than Leydig cell function, as revealed by low AMH and inhibin and nearly normal testosterone.

TUESDAY, APRIL 5, 2016
10:45 a.m. – 11:15 a.m.

SYMPOSIUM V – Male Reproductive Tract/Sperm Biology
EXPLORING THE ROLE OF MACROPHAGES AND DENDRITIC CELLS IN THE EPIDIDYMIS
Nicolas Da Silva, PhD
Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

The epididymis, a long and complex tube located downstream of the testis, is the organ in which sperm acquire motility and the ability to interact with an oocyte. The epididymis faces a unique immunological challenge: sperm, which express antigens that are not found elsewhere in the body, begin to populate the reproductive tract in abundance long after the establishment of central tolerance. Therefore, a finely regulated crosstalk between the immune system and the post-testicular environment is necessary to protect vulnerable spermatozoa against pathogens and autoimmunity. We have shown previously that the mouse epididymis contains a dense network of mononuclear phagocytes. Various subsets of macrophages and dendritic cells (DCs) line the duct and populate the interstitium. Recent advances have revealed that macrophages are shaped by their environment and continuously adapt to the need of their client tissues to control their development and homeostasis. Our project aims to elucidate the functions of DCs and macrophages in this intriguing yet poorly studied mucosal system.
Epididymal macrophages expressing CX3CR1, CD11c and F4/80 are actively involved in the homeostasis of the "blood-epididymis barrier" by rapidly removing apoptotic epithelial cells. In addition, macrophages from the most proximal region of the epididymis (initial segment, IS) project numerous dendrites toward the luminal compartment. We micro-inoculated labeled particles and soluble antigens into the lumen of the IS to study antigen uptake and processing *in situ.* Our results show that epididymal macrophages take up luminal antigens and occasionally sperm fragments. Furthermore, captured antigens were found in a subset of DCs expressing CD11c and CD103 that reside in the peritubular region. We will discuss our most recent results about antigen uptake by epididymal macrophages and DCs, as well as interactions with blood and lymphatic vessels. Epididymis resident macrophages and DCs contribute to the creation of a unique and dynamic immunological microenvironment that is a potential key regulator of male reproductive health.
Funding: Our work is supported by NIH/NICHD grant R01HD069623.

TUESDAY, APRIL 5, 2016
11:15 a.m. – 11:45 a.m.

SYMPOSIUM V - Male Reproductive Tract/Sperm Biology
Y CHROMOSOME AND SPERMATOGENESIS
Monika A. Ward, PhD
Institute for Biogenesis Research, John A. Burns School of Medicine, University of Hawaii

The mammalian Y chromosome encodes a battery of genes, many of which are thought to be involved in male reproduction. A substantial amount of work has been done to define which genes are important for maintaining sperm function under normal, in vivo, conditions. In the era of assisted reproduction technologies (ART), however, it is possible to bypass several steps of normal fertilization using immotile, nonviable or even immature sperm. Consequently, in the context of ART, the essential roles of the Y chromosome genes may become abrogated. Few years ago we have shown that live offspring can be obtained from mice lacking the entire Y chromosome long arm, in which the Y chromosome was reduced from 89.5 Mb to ~3.5 Mb and encoded only nine genes and three gene families. Subsequently, we demonstrated that offspring can also be generated from males with the Y chromosome contribution limited to only two genes, the testis determinant *Sry* and the spermatogonial proliferation factor *Eif2s3y*. These males generated haploid round spermatids, but not sperm. We have now

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identified a third gene, which when added to *Sry* and *Eif2s3y* allows for sperm formation and successful intracytoplasmic sperm injection (ICSI) in the absence of Y chromosome genes. We also have evidence showing that the function of *Sry* and *Eif2s3y* can be replaced by that of other genes, encoded on chromosomes other than Y and that live offspring can be obtained from male mice without any Y chromosome DNA. The progress in our investigations of Y chromosome gene function will be presented at the meeting.

Funding: Funding provided by NIH HD072380 and HCF 14ADVC-64546.

TUESDAY, APRIL 5, 2016
11:45 am – 12:15 p.m.

SYMPOSIUM V - Male Reproductive Tract/Sperm Biology

"BREAKING" NEWS FROM SPERMATIDS

Guylain Boissonneault, Olivier Simard and Marie-Chantal Grégoire
Department of Biochemistry, Faculty of Medicine & Health Sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada

DNA double-strand breaks (DSBs) are considered a serious threat to genome integrity since they are potent inducers of inappropriate recombination, mutation and even cell death. Genetic alterations resulting from DSBs are expected to be especially deleterious in haploid germ cells since DNA repair by homologous recombination cannot be used due to the lack of sister chromatids. Haploid spermatids undergo a dramatic differentiation program and a major remodeling of the chromatin where most of the histones are lost and replaced by protamines. Using neutral comet assay, pulse field gel electrophoresis, DNA end-labeling as well as immunofluorescence, we have established that the chromatin remodeling steps in the mouse model display a transient surge in DSBs while repair proceeds towards the final steps of spermiogenesis. Without the possibility of templated DNA repair, DSBs repair in spermatids should rely mostly on DNA end-joining mechanisms known to induce short insertions or deletions (indels). Although very challenging, we proceeded to set up a method for the genome-wide mapping of DSBs using next generation sequencing so as to establish the genomic distribution of DSBs and study mutagenesis at potential hotspots and through the differentiation steps. Given the significant number of DSBs that are found, we surmised that an alternative mechanism may exist in order to attenuate the potential mutagenicity of these DSBs. Recently, a new mechanism involving RNA-templated homologous recombination (RTHR) has been shown to improve repair fidelity in yeast so we sought to determine whether spermatids may rely on a similar mechanism. We generated an active nuclear extract from elongating and condensing spermatids and showed that an *in vitro* synthesized, fully complementary RNA transcript can indeed be used to repair a plasmid DNA fragment harboring a sequence gap generated by a double digestion with restriction enzymes. Use of the template RNA sequence was also confirmed by incubating a complementary transcript, harboring a central heterologous sequence that showed productive recombination within the gap. These results suggest that RTHR may represent an alternative mechanism to increase the fidelity of DSBs repair in spermatids. Because we have shown that the transient DSBs and repair is conserved from mouse to human, and is also observed during sporulation in yeast, the transient DSBs and repair process deserves further investigation as a critical mechanism that may impact genetic integrity in the male gamete and contributes to the male mutation bias.

Funding: Funded by the Canadian Institutes of Health Research and a Scholarship from FRQS (Quebec) to O.S.

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ORAL ABSTRACTS

Sunday, April 3, 2016
2:00 p.m. – 3:30 p.m.

Concurrent Oral Session I: Basic Science
Location: Grand Ballroom CD
Moderator: Robert S. Viger, PhD

1

SERTOLI CELL GENE THERAPY IN SERTOLI CELL ANDROGEN RECEPTOR KNOCKOUT (SCARKO) MICE

Chatchanan Doungkamchan, MD[1], Yi Sheng, MD, PhD[2], Meena Sukhwani, PhD[3], Robert E. Braun, PhD[4] and Kyle E. Orwig, PhD[2]
[1]Molecular Genetics and Developmental Biology Graduate Program, Magee-Womens Research Institute, University of Pittsburgh School of Medicine; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Magee-Womens Research Institute, Pittsburgh, PA; [4]The Jackson Laboratory, Bar Habor, ME
(Presented By: Chatchanan Doungkamchan, MD)

Introduction: Non-obstructive azoospermia with a uniform maturation arrest phenotype (NOA-MA) has poor prognosis for sperm retrieval by testicular sperm extraction (TESE). Some cases of NOA-MA can be linked to a single gene defect in somatic cells and/or Germ cells. We hypothesize that gene therapy can restore aberrant gene expression and fertility in some men with NOA-MA. Since the ethics of germline gene therapy are actively debated, our objective was to provide a proof in principle that gene therapy can correct a defective gene in Sertoli cells without germline modification. We used non-integrating adenoviral vectors to deliver a human androgen receptor (hAR) transgene to Sertoli Cell Androgen Receptor Knockout (SCARKO) mice, which have an NOA-MA phenotype.
Methods: The therapeutic vector, Adeno-hAR, contains an EF1a promoter driving EGFP and hAR expression. The Adeno-empty control vector did not have hAR. To evaluate transgene delivery, we injected Adeno-empty (2.8x106 ifu) into the seminiferous tubules of one testis. The injected and non-injected testes were evaluated 20 days later by co-staining for EGFP/VASA (germ cell marker) or EGFP/SOX9 (Sertoli cell marker). To evaluate if Adeno-hAR could restore AR expression, we injected the right testis of SCARKO mice with Adeno-hAR (2.8x106 ifu) and the left testis with Adeno-empty (2.8x106 ifu). Immunohistochemistry was done to determine EGFP and AR expression 20 days after injection.
Results: SCARKO mice were infertile; had small testes with an NOA-MA phenotype and lacked AR expression in Sertoli cells. The adenoviral vectors efficiently transduced SOX9+ Sertoli cells, but not VASA+ germ cells. Adeno-hAR restored AR expression in Sertoli cells of SCARKO mouse testes. Adeno-empty did not.
Conclusion: Sertoli cell gene therapy may have application for treating some cases of male infertility. We confirmed that non-integrating adenoviral vectors could introduce a corrective hAR transgene into Sertoli cells. Ongoing studies will quantify the risk of germline modification, map viral integration sites and evaluate fertility of untreated versus treated mice by breeding.
Funding: Magee-Womens Research Institute and Foundation and grants from the Eunice Kennedy Shriver National Institute for Child Health and Human Development (HD076412 and HD075795).

2

GLYCOLYSIS OPTIMIZED CONDITIONS IMPROVE THE REGENERATIVE QUALITY OF SPERMATOGONIAL STEM CELLS DURING LONG-TERM IN VITRO MAINTENANCE

Aileen Helsel and Jon Oatley, PhD
Washington State University
(Presented By: Aileen Helsel)

Introduction: Spermatogonial stem cells (SSCs) are a rare subset of the undifferentiated spermatogonial population and their activities provide the foundation for continual sperm production. A remarkable feature of SSCs is their capacity to regenerate the spermatogenic lineage following transplantation into the testes of a sterile recipient. A potential clinical application of SSC transplant is to treat infertility in cancer patients with germline elimination caused by cytotoxic effects of chemotherapy. The number of SSCs that can be collected from testicular tissue is low, thus, application is dependent on the ability to expand a primary population in vitro.
Methods: At present, culture methodologies are available for rodents and are being used as the basis for development of similar conditions for human SSCs. However, the percentage of rodent primary culture populations that possess regenerative capacity is low (1-10%) and the ability to regenerate complete colonies of spermatogenesis declines during long-term culture (4-6 months). Thus, there is need to refine culture conditions to optimize SSC maintenance and a potential avenue to address this is to match conditions to SSC bioenergetics.
Results: Based on the outcomes of RNA-Seq analysis, we discovered that expression of key genes involved in the regulation of glycolysis are up-regulated in SSCs. In the current study, we explored the impacts of altering culture conditions to favor utilization of glycolysis as the primary bioenergetics process on maintenance of SSCs in primary cultures of mouse undifferentiated spermatogonia. Conventional culture conditions include maintenance in 21% O2 and media containing free fatty acids, which promote utilization of beta-oxidation as the primary bioenergetics process. To produce glycolysis optimized (GO) conditions, we cultured cells in 10% O2 and removed free fatty acids from the media. Transplantation analyses at two-month intervals revealed that the capacity to regenerate complete colonies of spermatogenesis was improved over the six-month culture period in the GO condition compared to the conventional condition.
Conclusion: These findings indicate that matching culture conditions to the bioenergetics effects stem cell fidelity in primary cultures of mouse undifferentiated spermatogonia. Importantly, this information may be useful in devising culture conditions for SSCs of higher order mammals including humans. This research was supported by grant HD061665 awarded to JMO from the NICHD.

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ORAL ABSTRACTS

3

THE TYROSINE KINASE FER IS RESPONSIBLE FOR THE CAPACITATION-ASSOCIATED INCREASE IN TYROSINE PHOSPHORYLATION

Antonio Alvau, MSc and Pablo Visconti, Principal Investigator
University of Massachusetts, Amherst
(Presented By: Antonio Alvau, MSc)

Introduction: Sperm capacitation comprises all the physiological changes occurring to mammalian spermatozoa during the transit through the female reproductive tract and is necessary for successful fertilization both in vivo and in vitro. At the molecular level, the process is associated with a fast activation of cAMP-dependent phosphorylation by protein kinase A (PKA). The activation of PKA leads to a slow increase in the levels of tyrosine phosphorylation, another established hallmark of sperm capacitation. Several candidates to be responsible for the increase in tyrosine have been suggested. However, due to limitations in the use of knock-out approaches for sperm research, the identity of the kinase ultimately responsible for the increased levels of phosphorylation has not been demonstrated yet.
Objective: The aim of the present research was to investigate the identity of the tyrosine kinase responsible for the capacitation-induced increase in tyrosine phosphorylation.
Methods: In order to fulfill this purpose, both pharmacological inhibitors and genetically modified murine models were used. Levels of Tyrosine phosphorylation were tested by SDS-page, with antibodies directed against phosphorylated tyrosine residues. Effect of pharmacological inhibitors on recombinant kinases in vitro was tested by the use of radioactive assay.
Results: Mice lacking PYK2 tyrosine kinase display normal capacitation-associated increase in tyrosine phosphorylation. On the other hand, sperm from mice targeted with a mutation that abolish the activity of the FER kinase (FERDR/DR), fail to undergo the increase in tyrosine phosphorylation associated with sperm capacitation. Our results indicate that FER tyrosine kinase is the tyrosine kinase responsible for the capacitation-associated increase in tyrosine phosphorylation. Interestingly, although sperm from FERDR/DR animals have significantly reduced capability to fertilize metaphase II-arrested eggs in vitro, these mice are fertile in vivo.
Conclusion: Our results suggest that FER is the kinase responsible for the regulation of the increase of tyrosine phosphorylation associated with sperm capacitation in murine.

4

LACK OF MEMBRANE ESTROGEN RECEPTOR ALPHA LEADS TO SPERM ABNORMALITIES, DECREASED SPERM MOTILITY AND VIABILITY, AND PROGRESSIVE INFERTILITY IN MALE MICE

Manjunatha Nanjappa, PhD[1], Theresa Medrano, MS[1], Seth Locker[1], Rex Hess, PhD[2], Ali Pedram, PhD[3], Ellis Levin, MD[4] and Paul Cooke, PhD[1]
[1]University of Florida, Gainesville, FL; [2]University of Illinois at Urbana-Champaign, Urbana, IL; [3]University of California, Irvine, CA; [4]University of California, Irvine, CA and Department of Veterans Affairs Medical Center, Long Beach, CA
(Presented By: Manjunatha K. Nanjappa, BVSc & AH, MVSc, PhD)

Introduction: 17β-Estradiol (E2) regulates male and female reproduction through its main receptor, estrogen receptor alpha (ESR1). ESR1 is expressed in rete testes, efferent ductules (EDs) and epididymis, and global loss of ESR1 (Esr1KO) causes infertility due to failure of EDs to concentrate testicular fluid, resulting in back pressure atrophy of seminiferous tubules (ST) in mice. ESR1 is mainly nuclear/cytoplasmic; however, 5% of ESR1 is palmitoylated at cysteine 451 in mice and localized to cell membranes, where it mediates rapid E2 actions.
Method: This study investigated roles of membrane ESR1 (mESR1) in male reproduction using Esr1 knockin mice containing a mutated palmitoylation site (C451A) in ESR1 which prevented cell membrane localization; these nuclear-only estrogen receptor 1 (NOER) mice express nuclear ESR1 (nESR1) but lack mESR1. Reproductive organ weights and histology, daily sperm production (DSP), sperm morphology and motility, hormones and breeding were analyzed in 4- and 8-month-old wild-type (WT) and homozygous NOER male mice.
Results: Testes weights of NOER mice were significantly higher than controls at 4 months, but not different at 8 months. NOER testes had increased numbers of abnormal STs; this was more severe at 8 months than 4 months. Rete testes of NOER mice were dilated compared to WTs. At 8 months, DSP was reduced 62% in NOER mice compared to WTs. Epithelial height of EDs was reduced and seminal vesicle weights increased in NOER vs WT mice. Caudal epididymal sperm from NOER mice showed a 75% decrease in total and progressive motility and a 40% decrease in viability compared to WT mice at 8 months of age. Furthermore, >95% of NOER epididymal sperm exhibited morphological defects (e.g., folded or coiled tail and/or absent head) compared to WT; these defects were not seen in testis, indicating they resulted from an altered epididymal environment. Serum testosterone was increased in NOER mice versus WT mice at 4 months while LH, FSH and E2 were not different.
Conclusion: Young NOER males (6 to 12 weeks) were initially subfertile, but infertile by 5 months. Loss of mESR1 in male mice causes impaired sperm production, motility and viability, extensive sperm morphological defects and progressive infertility. These results demonstrate that membrane initiated E2/ESR1 signaling is required for normal testicular and reproductive tract function and fertility but how mESR1 and nESR1 cooperate to mediate overall E2 effects on male reproduction remains to be established.

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ORAL ABSTRACTS

5

LONG-TERM BRDU LABELING IDENTIFIES MITOTICALLY ACTIVE ADARK AND APALE SPERMATOGONIA IN ADULT RHESUS MACAQUE TESTES

Adetunji Fayomi, DVM, MVSc[1], Brian P. Hermann, PhD[2], Gary R. Marshall, PhD[3] and Kyle E. Orwig, PhD[4]
[1]Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine; [2]Department of Biology, University of Texas San Antonio; [3]Department of Natural Sciences, Chatham University; [4]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine
(Presented By: Adetunji Fayomi)

Introduction: This study challenges the dogma that Adark and Apale spermatogonia function as "reserve" and "active" stem cells during steady state spermatogenesis in adult primate testes. We hypothesize that Adark are slow cycling "active" stem cells and that this will be revealed by long-term mitotic labeling with BrdU.
Methods: BrdU was administered to adult Rhesus macaques as a single intravenous bolus or administered continuously in the drinking water for three weeks. Testes were collected for analysis three hours after the bolus of BrdU or zero, five or nine weeks after the continuous administration.
Results: In testes collected three hours after a single bolus of BrdU, 2.5% of Adark and 28% of Apale were labeled, consistent with previous reports. From those data, it is difficult to ascertain whether the low level of Adark labeling is artifact or represents the expected low level labeling of slow cycling stem cells. Therefore, we administered BrdU continuously in the drinking water for three weeks and observed that 14% of Adark and 69% of Apale spermatogonia incorporated label. These results indicate that at least a portion of Adark spermatogonia are mitotically active in adult primate testes. Similarly, at least a portion of Apale spermatogonia are relatively quiescent. After five weeks of washout, the proportion of labeled Adark increased to 24% while the proportion of labeled Apale decreased to 29%. It is not possible to determine whether the increase in labeled Adark after five weeks of washout is due to subsequent divisions of labeled Adark; reversion of labeled Apale to Adark or both. The reduction in labeled Apale between zero and five weeks of washout is presumably due to differentiation and possibly reversion to Adark. Finally, Adark and Apale labeling was reduced nearly to zero after nine weeks of washout. These results indicate that the cells that incorporated label continued to be mitotically active and/or differentiate during the 12 week course of the experiment.
Conclusion: This long-term BrdU labeling experiment allowed us to unequivocally demonstrate that Adark are mitotically active in steady state spermatogenesis. Therefore, it is necessary to revisit the generalized designation of Adark and Apale spermatogonia as "reserve" and "active" stem cells in the adult primate testis.
Funding: This work was supported by NICHD grants HD055475, HD076412 and HD075795 and the Magee-Womens Research Institute and Foundation.

6

PROTEIN TYROSINE KINASE 7 SIGNALS THROUGH MYOSIN II TO REGULATE PLANAR CELL POLARITY-DEPENDENT TUBULAR MORPHOGENESIS OF THE WOLFFIAN/EPIDIDYMAL DUCT, WHICH IN TURN IS IMPORTANT FOR THE ACQUISITION OF SPERM MOTILITY

Bingfang Xu, PhD[1], Angela M. Washington, BS[1], Raquel Fantin-Domeniconi, PhD[2], Ana Cláudia Ferreira-Souza, MS[3], Xiaowei Lu, PhD[1], Sutherland Ann, PhD[1] and Barry T. Hinton, PhD[1]
[1]University of Virginia; [2]Institute of Biosciences – UNESP, Botucatu, Brazil; [3]Federal University of Viçosa, Viçosa, Brazil
(Presented By: Bingfang Xu, PhD)

Introduction: The Wolffian duct undergoes extensive morphogenesis to achieve an optimal length and size for sperm maturation. This process is not trivial because it results in a 1 m epididymal duct in the mouse, 3 m in the rat and 6 m in the human.
Method: To uncover the events leading to normal morphogenesis and to examine the cellular mechanisms that regulate these events, we used a conditional knockout approach to remove protein tyrosine kinase 7 (Ptk7), a regulator of planar cell polarity (PCP) and a gene previously shown to regulate cochlea and neural plate morphogenesis, from the mesoderm (Wolffian duct epithelium and surrounding mesenchyme).
Results: Our studies showed that the Wolffian duct was characterized as: (1) having epithelial cells that divided in a random orientation relative to the elongation axis of the duct, (2) the epithelium being planar polarized, which is characterized by oriented cell elongation at basal ends and oriented cell-cell association between apical and basal ends, and polarized activity of regulatory light chain of myosin II, (3) elongating via convergent extension-type of cell rearrangements while maintaining a relatively unchanged duct diameter. Loss of Ptk7 from the mesoderm resulted in loss of all PCP characteristics and failure of cells to undergo convergent extension rearrangements resulting in a duct that had shortened length, increased lumen diameter and reduced coiling that eventually led to loss of sperm motility. However, Ptk7 did not regulate cell division nor division orientation. Furthermore, deletion of Ptk7 from mesenchymal cells surrounding the epithelium did not affect duct elongation and coiling. However, deletion of Ptk7 from epithelial cells resulted in a similar phenotype to removal of Ptk7 from the mesoderm suggesting epithelial PTK7 autonomously regulates epithelial PCP in the Woffian duct.
Conclusion: Our data supports the hypothesis that PTK7 signals through myosin II to regulate PCP to ensure that convergent extension cell rearrangements drive elongation and coiling of the Wolffian duct. Therefore, PTK7 is important for Wolffian duct morphogenesis and male fertility.
Funding: This work was supported by Eunice Shriver National Institute of Child Health and Human Development/NIH Grant RO1-HD069654 (BTH), R01-DC013773 (XL), FAPESP-2014/23382-9 (RFD) and CAPES-BEX 5874/14-9 (ACFS).

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ORAL ABSTRACTS

Sunday, April 3, 2016
2:00 p.m. – 3:15 p.m.

Concurrent Oral Session II
Location: Grand Ballroom AB
Moderator: Arthur Burnett II, MD, MBA

7

ANDROGEN RECEPTOR DEGRADATION BY SULFORAPHANE ENHANCES THE EFFICACY OF ANTI-ANDROGENS AGAINST AGGRESSIVE PROSTATE CANCER CELLS

Namrata Khurana, PhD[1], Sudha Talwar[1], Partha Chandra[2], Asim Abdel-Mageed[1], Debasis Mondal[2] and Suresh Sikka[1]
[1]Department of Urology, Tulane University; [2]Department of Pharmacology, Tulane University School of Medicine
(Presented By: Namrata Khurana, PhD)

Introduction: Androgen deprivation therapy (ADT) remains the treatment of choice for prostate cancer (PC) since PC cells utilize androgen receptor (AR) for their survival and proliferation. Although initially effective, the tumor suppressive effects of anti-androgens, e.g. Bicalutamide (BIC) and Enzalutamide (ENZ), are only transient and PC cells eventually become resistant to ADT to enable the outgrowth of Castration Resistant Prostate Cancer (CRPC). Therefore, alternative approaches to downregulate AR expression in PC cells are needed to augment the efficiency of anti-androgens. Sulforaphane (SFN), a phytochemical derived from broccoli, is known to destabilize AR. We hypothesized that simultaneous exposure to physiologically achievable concentrations of SFN would be able to sensitize PC cells to antiandrogen-induced growth suppression.
Methods: In both androgen dependent (LNCaP) and androgen independent PC cell lines (C4-2B) that express AR, we monitored the anticancer effects of BIC or ENZ in the presence or absence of SFN. Effect of drug(s) on cell growth and colonization were monitored by MTT-assays and Colony Forming Unit (CFU) assays, respectively. AR expression was measured by immunoblot analysis (IB), and its subcellular localization was measured by immunofluorescence microscopy (IFM). The expression of AR and PSA (prostate specific antigen) mRNA was determined by quantitative RT-PCR.
Results: The MTT and CFU assays revealed that co-exposure to SFN significantly ($p<0.05$) enhances the growth suppressive effect of BIC and ENZ in both LNCaP and C4-2B cells. This drug combination was also able to significantly ($p<0.005$) suppress the mitogenic effects of an androgen agonist (R1881). The IB analysis showed that SFN induces degradation of AR protein in a time- and dose-dependent manner. Within 24 hours post-exposure, SFN (10 μM) exposure could decrease AR protein levels by almost 75%. RT-PCR analysis showed that SFN enhances the efficacy of BIC in decreasing AR and PSA gene expression. The IFM studies also indicated that SFN suppresses both total AR levels and the nuclear translocation of AR following R1881 stimulation of PC cells.
Conclusion: Our findings demonstrate that co-exposure to SFN can sensitize the AR expressing PC cells to antiandrogens by rapidly degrading AR protein levels. Therefore, this safe phytochemical may be used as an adjuvant therapy with ADT to suppress the recurrence of CRPC.

8

CYSTEINE-RICH SECRETORY PROTEIN 3 (CRISP-3) IS A CANDIDATE BIOMARKER OF TESTICULAR DYSFUNCTION IN ADOLESCENTS WITH VARICOCELE

Larissa Berloffa Belardin, BSc, Mariana Camargo, MSc, Paula Intasqui Lopes, MSc, Paula Toni Del Giudice, PhD, Daniel Suslik Zylbersztejn, MD, PhD, Ricardo Pimenta Bertolla, DVM, PhD and Agnaldo Pereira Cedenho, MD, PhD
Department of Surgery, Division of Urology, Universidade Federal de São Paulo
(Presented By: Larissa Berloffa Belardin, BSc)

Introduction: Varicocele, the presence of varicose veins in the pampiniform plexus with retrograde blood flow, initiates at puberty onset. Although many studies have associated varicocele to a progressive decline in male fertility, mainly due to testicular hyperthermia and decreased removal of toxic metabolites, underlying molecular mechanisms are not understood. In fact, adolescents with varicocele may or may not present infertility in adulthood. We have previously demonstrated alterations in the seminal plasma proteome in the presence of varicocele-induced testicular dysfunction in adolescents. We wished to validate these proteins to verify their potential in separating an infertility-defining varicocele from a "silent" varicocele. Thus, our objective was to verify seminal levels of Deoxyribonuclease 1 (DNase I), Ig gamma-3 chain C region (IGHG3), Lipid phosphate phosphohydrolase 1 (PAP2-alpha), 45 kDa calcium binding protein (Cab45), and Cysteine-rich secretory protein 3 (CRISP-3) in adolescents with and without varicocele.
Methods: 61 adolescents, between 15-19 years old, with full sexual maturity, were divided into three groups: controls without varicocele (C); with varicocele and normal semen (VNS); with varicocele and altered semen (VAS). Samples were centrifuged and the supernatant seminal plasma collected. Thenceforth, 50 μg of protein was utilized for Western blotting for quantification of DNase I, IGHG3, PAP2-alpha, Cab45, and CRISP-3 (normalized to Epididymis Secretory Sperm Binding Protein Li 67p [DJ-1]). Groups were compared using ANOVA (post hoc Sidak) or Kruskal-Wallis (post hoc Games Howell) ($p<0.05$).
Results: Results are presented in Table 1. VAS patients presented lower sperm concentration and morphology. DNase I was underexpressed in the VNS group, Cab45 was underexpressed in both varicocele groups, and CRISP-3 was overexpressed in the VAS group.
Conclusion: The seminal plasma proteome reflects alterations in testicular function in the adolescent varicocele. CRISP-3 is a candidate biomarker of testicular dysfunction in adolescents with varicocele, and in the future may assist in determining medical conduct for early surgical intervention.
Support: São Paulo Research Foundation (2014/11636-6).

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Table 1. Semen analysis and seminal plasma levels of DNase1, IGHG3, PAP2-Alpha, Cab45 and CRISP3. Groups were compared by ANOVA followed by a Sidak *post-hoc test* (values presented as mean; standard deviation), unless otherwise noted.

	C (n=20)	VNS (n=22)	VAS (n=19)	P
Age (years)	16.5; 2.00	16.0; 2.00	16.0; 1.00	[D] 0.595
Abstinence (days)	3.5; 1.40	4.0; 2.00	3.0; 2.00	[D] 0.289
Sperm concentration (x10^6/mL)	80.5; 50.49 [a]	96.1; 60.94 [a]	13.4; 11.25 [b]	<0.00001 *
Progressive motility (%)	64.5; 10.50	58.0; 10.00	56.0; 16.00	[D] 0.122
Total motility (%)	65.5; 7.25	60.1; 12.18	60; 12.65	0.201
Morphology (% normal)	9.5; 3.80 [a]	9.0; 4.00 [a]	2.0; 4.00 [b]	[D] <0.00001 *
DNase I (a.u.)	1.8; 2.29 [a]	0.2; 0.33 [b]	1.9; 2.64 [a]	0.013 *
IGHG3 (a.u.)	1.1; 0.81	2.5; 6.36	1.4; 1.39	0.814
PAP2-Alpha (a.u.)	0.9; 1.99	0.8; 0.93	0.8; 1.04	0.789
Cab45 (a.u.)	1.4; 2.34 [a]	0.2; 0.25 [b]	0.1; 0.16 [b]	0.007 *
CRISP-3 (29KDa) (a.u.)	1.7; 1.96 [a]	1.3; 1.73 [a]	84.1; 94 [b]	<0.00001 *
CRISP-3 (31KDa) (a.u.)	2.3; 2.06 [a]	2.6; 5.36 [a]	187.8; 344.96 [b]	<0.00001 *

[D] – Nonparametric Kruskal-Wallis test, Games Howell *post hoc* test. Values expressed in median; interquartile range (IR).
* – statistically significant difference.
Different superscript letters in a same row indicate a significant difference in the *post-hoc* test.
(a.u.) – arbitrary units, normalized to the testicular housekeeping protein DJ-1.

9

IFT140 IS A NOVEL CANDIDATE GENE FOR IMPAIRED SPERMATOGENESIS: IDENTIFICATION BY WHOLE EXOME SEQUENCING AND VALIDATION WITH SANGER SEQUENCING

Amin Herati, MD, Peter Butler, BS, Cenk Cengiz, BS, Matthew Bainbridge, PhD, James Lupski, MD, PhD, Richard Gibbs, PhD, Shalini Jhangiani, MS, Larry Lipshultz, MD and Dolores Lamb, PhD
Baylor College of Medicine
(Presented By: Amin S. Herati, MD)

Introduction: One-third of male factor infertility cases are idiopathic and are likely caused by genetic factors. Non-obstructive azoospermia (NOA) is characterized by the absence of sperm in the ejaculate secondary to the lack of sperm production in the testicle. The aim of our study was to identify novel variants implicated in the pathogenesis of NOA through whole-exome sequencing (WES).
Methods: Homozygosity mapping with WES was performed using the NimbleGen SeqCap EZ Exome V3 sample preparation kit and the Illumina HiSeq 2000 next-generation sequencing platform to sequence the exomes of two siblings with NOA from a consanguineous family. Genetic mutations that passed filter criteria were validated using Sanger sequencing. We identified one candidate gene, Intraflagellar Transport Protein 140 (IFT140), based on its expression pattern in testes according to the Human Protein Atlas, as well the mutation's damage prediction based on two online bioinformatics resources. We subsequently performed Sanger sequencing on 114 NOA and 43 fertile men.
Results: WES identified 442 variants in the index patients. Four (0.9%) of these variants passed filter criteria, one of which was a six-nucleotide deletion (HG38, chr16:1,519,961 TCTTGGC>T) in exon 22 of IFT140. Family segregation showed that this mutation was homozygous in the two brothers who underwent WES, as well as in a third brother with NOA and their sister. This mutation was not identified in the 1000 Genome Project and was predicted to be deleterious by the bioinformatics tools, MutationTaster and PROVEAN. The Exome Aggregation Consortium database of 60,706 genomes was also queried which identified a minor allele frequency of 0.1%. Sanger sequencing validated the mutation in this family and identified it as a heterozygous mutation in 1 (0.9%) of the 114 NOA men and none of the 43 fertile controls in our cohort. Testis biopsy interpretation of this unrelated patient showed hypospermatogenesis with a clinical history significant for three failed cycles of in vitro fertilization (IVF).
Conclusion: We identified a small indel mutation in IFT140 as a novel candidate variant for infertility. This mutation was detected in our cohort of NOA men in addition to the index family, with a prevalence of 0.9%, which is higher than the minor allele frequency in an unselected general population. Further studies are required to determine the role of IFT140 in spermatogenesis.

10

CASANOVA: A MULTICLASS SUPPORT VECTOR MACHINE MODEL FOR THE CLASSIFICATION OF HUMAN SPERM MOTILITY PATTERNS

Summer Goodson, PhD[1], Sarah White[2], Alicia Stevans[3], Sanjana Bhat[3], Chia-Yu Kao[2], Scott Jaworski[1], Tamara Marlowe[1], Martin Kohlmeier, MD, PhD[1], Leonard McMillan, PhD[2], Steven Zeisel, MD, PhD[1] and Deborah O'Brien, PhD[3]
[1]University of North Carolina at Chapel Hill Nutrition Research Institute; [2]Department of Computer Science, University of North Carolina at Chapel Hill; [3]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill
(Presented By: Summer Goodson, PhD)

Introduction: Hyperactivated motility in sperm is required for normal fertilization. Traditionally, the percentage of hyperactivated human sperm assessed by computer-assisted sperm analysis (CASA) has been reported based on threshold gates for multiple kinematic parameters that reflect hyperactivated motility patterns. We previously reported a novel CASA-based approach, CASAnova, for identifying multiple patterns of sperm motility in mouse. This approach uses multiple support vector machine (SVM) equations based on standard CASA parameters to characterize the distribution of motility patterns in large populations of sperm. We have now adapted CASAnova for the analysis of human sperm motility.
Methods: Using 2,817 visually classified sperm tracks obtained from 14 individuals, we generated a web-based program that incorporates kinematic parameters of individual sperm into five SVM equations. These equations are used to classify sperm tracks into one of five categories of motility (progressive, intermediate, hyperactivated, slow and weakly motile) with an overall accuracy exceeding 89%.
Results: Comparison of visually-assessed levels of hyperactivation versus levels generated by CASAnova or kinematic thresholds shows that CASAnova estimates of hyperactivated motility more closely reflect visual estimates than those calculated by gating. When applied to populations of sperm incubated in capacitating vs. non-capacitating conditions, CASAnova accurately detects the absence of hyperactivated motility in non-capacitated sperm, as well as a reduction in intermediate and progressive motility. These results indicate that CASAnova accurately detects changes in sperm motility when signaling pathways controlling normal sperm capacitation are perturbed. Furthermore, motility profiles of sperm from men harboring two minor alleles of the single nucleotide polymorphism rs12676 (located in the gene encoding choline dehydrogenase), reveal a delay in the onset of hyperactivation, a phenotype than cannot be seen using traditional gating. CASAnova allows for robust, accurate and detailed analyses of sperm motility patterns. The sensitivity of this method facilitates the detection of defects that are not seen with traditional approaches for assessing sperm motility, indicating its potential utility in future clinical applications that evaluate male infertility.

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11

THE EFFECT OF ORCHIECTOMY ON FUNCTIONAL ASPECTS OF SPERM AND OXIDATIVE STRESS OF SEMINAL PLASMA FROM PATIENTS WITH TESTICULAR TUMORS

Maria Beatriz Ribeiro de Andrade, Paula Intasqui, Larissa Belardin, Mariana Antoniassi, Danielle Tibaldi, Ricardo Pimenta Bertolla and Deborah Spaine
Sao Paulo Federal University
(Presented By: Maria Beatriz Ribeiro De Andrade, BSc)

Introduction: Testicular tumors, although rare, are the most frequent tumors in young men of reproductive age. Treatment is usually achieved by orchiectomy, followed or not by adjuvant therapy, with survival rates of 95% after five years. Because adjuvant therapy is often gonadotoxic, there is a concern with reproductive life. Currently, sperm banking is the only option that guarantees future fertility in these men. However, it remains to be answered if the best sample to cryopreserve is the pre- or the post-orchiectomy sample. Therefore, we wished to verify semen quality, sperm functional traits, and semen oxidative stress, before and after orchiectomy and suggest the better time to perform sperm banking in patients with testicular tumors.
Methods: This prospective study was carried out including 24 patients with testicular germ cell tumors, who provided one semen sample before orchiectomy of the affected testis and one at least 15 days after the surgery. Following semen analysis, an aliquot was used for analysis of (i) sperm DNA fragmentation (alkaline Comet assay, sperm were classified as high DNA integrity [Class I] to high DNA fragmentation [Class IV]); (ii) acrosome integrity (PNA-FITC); and (iii) mitochondrial activity (colorimetric staining of the midpiece, classified as Grade I [all mitochondria active] to IV [all mitochondria inactive]. Seminal plasma MDA levels were measured as markers of oxidative stress. Pre- and Post-orchiectomy samples were compared using a paired Student's T test (normal distributions) or a paired Wilcoxon test, when appropriate ($p<0.05$).
Results: Results are presented in table 1. A significant decrease in DNA fragmentation ($p=0.019$) and oxidative stress ($p=0.014$), and an increase in mitochondrial activity ($p= 0.048$) was observed after orchiectomy.
Conclusion: Based on our findings, the better time for sperm banking in patients with testicular tumors is after orchiectomy. Although cryopreservation of pre-orchiectomy samples remains an important conduct to preserve reproductive potential, post-orchiectomy samples should be preferred for assisted reproductive treatments.

Table 1. Value of seminal analysis and DNA fragmentation (Comet), mitochondrial activity (DAB), acrosome integrity (PNA – FITC) and lipid peroxidation (TBARS). Value presented as mean; standard deviation.

	Pre - orchiectomy	Post - orchiectomy	p
Volume (mL)	3.0; 1.24	3.2; 2.46	0.620*
Progressive (%)	51.9; 13.03	50.7; 10.16	0.819
Non-progressive (%)	42.6; 12.76	44.8; 9.79	0.646
Concentration (x 10^6/mL)	26.7; 24.93	24.2; 27.12	0.227*
Morphology (%)	4.4; 2.50	4.4; 2.10	0.714
Comet I (%)	7.0; 11.00	6.5; 7.00	0.36
Comet II (%)	62.5; 12.00	72.0; 18.00	0.019*
Comet III (%)	22.5; 8.00	14.5; 9.00	0.028*
Comet IV (%)	5.0; 8.00	3.0; 5.00	0.172
DAB I (%)	1.0; 3.00	1.0; 3.00	0.612
DAB II (%)	65.5; 29.00	65.0; 17.00	0.075
DAB III (%)	34.2; 15.32	28.1; 7.71	0.048*
DAB IV (%)	4.5; 7.00	4.0; 4.00	0.702
Intact acrosomes (%)	80.9; 8.66	80.4; 8.39	0.521
MDA (ng/ml)	9.5; 4.38	7.8; 2.79	0.014*

* - Significant difference
* - Nonparametric Wilcoxon test. Value present as median; interquartile range.

12

CONSISTENT DIFFERENCES AMONG MEN IN CAPACITATION TIMING COULD PERSONALIZE IUI/ART

AJ Travis, VMD, PhD[1], C. Cardona, PhD[2], MA Moody[2], AJ Simpson[2], EK Seaman, MD[3] and GC Ostermeier, PhD[2]
[1]Cornell University, Androvia LifeSciences; [2]Androvia LifeSciences; [3]Urology Group of New Jersey
(Presented By: Alexander J. Travis, VMD, PhD)

Introduction: To become fertilization competent, sperm must first capacitate. This process depends on changes in membrane lipids, including removal of sterols and redistribution of the ganglioside GM1. Here, we examine the timing of capacitation among and within different individuals. Reliable differences in capacitation timing could be used to personalize IUI/ART protocols.
Methods: Semen samples from consenting men were liquefied, washed and aliquots incubated under non-capacitating (NC) or capacitating (CAP) conditions. Samples from fertile men (pregnant partner or recent father) and samples from men seeking fertility exams were incubated for three hours, fixed and analyzed immediately (day0) or after overnight incubation in fix (day1). In another trial, NC and CAP samples were incubated for three hours or 24 hours prior to fixation. Capacitation was assessed using localization of GM1 (Cap-ScoreTM).
Results: 102 samples from 36 fertile men were evaluated at day0 and day1. An increase in Cap-Score was observed in 81% (83/102) of CAP samples, with 44% (45/102) increasing more than 1 SD (7%). In 17 men seeking fertility treatment, 29% (5/17) increased 7% or more. To determine whether this change was physiological or an artifact of being in fix overnight, semen samples from nine fertile men were analyzed at day0, day1 and after 24 hours of incubation in medium and then fixed. All NC samples were equivalent (19±2, 23±2 and 20±1%) and were different from the CAP samples (28±1, 34±2 and 31±2%). Day1 CAP was significantly greater than Day0 CAP ($p=0.03$), but Cap-Scores for samples incubated overnight in fix or in capacitating media were the same ($p=0.33$). Consistent with prior literature, these data show that membrane changes involved in capacitation still occur in certain fixatives. These data suggest that sperm achieve capacitation at different times in different ejaculates. To see if this was reproducible for an individual, 91 samples from 25 fertile men were classified as either early or late capacitators (day1-day0>7). The average concordance of change within donors was 76%, showing that capacitation timing was highly consistent within men.
Conclusion: These data show that capacitation timing differed consistently among men. Determining capacitation rates could eventually lead to personalized management of infertility, including optimal times for IUI relative to ovulation and capacitation times prior to co-incubation of sperm and oocytes for IVF.
Funding: Funded by Androvia LifeSciences.

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POSTER SESSION I

Sunday, April 3, 2016
***Poster Session I**
11:00 a.m. – 12:30 p.m.
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

Poster #1
SERTOLI CELL GENE THERAPY IN SERTOLI CELL ANDROGEN RECEPTOR KNOCKOUT (SCARKO) MICE
Chatchanan Doungkamchan, MD[1]; Yi Sheng, MD, PhD[2]; Meena Sukhwani, PhD[3]; Robert E. Braun, PhD[4] and Kyle E. Orwig, PhD[2]
[1]Molecular Genetics and Developmental Biology Graduate Program, Magee-Womens Research Institute, University of Pittsburgh School of Medicine; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Magee-Womens Research Institute, Pittsburgh, PA; [4]The Jackson Laboratory, Bar Habor, ME
(Presented By: Chatchanan Doungkamchan, MD)

Poster #2
GLYCOLYSIS OPTIMIZED CONDITIONS IMPROVE THE REGENERATIVE QUALITY OF SPERMATOGONIAL STEM CELLS DURING LONG-TERM IN VITRO MAINTENANCE
Aileen Helsel and Jon Oatley, PhD
Washington State University
(Presented By: Aileen Helsel)

Poster #3
THE TYROSINE KINASE FER IS RESPONSIBLE FOR THE CAPACITATION-ASSOCIATED INCREASE IN TYROSINE PHOSPHORYLATION
Antonio Alvau, MSc and Pablo Visconti, Principal Investigator
University of Massachusetts, Amherst
(Presented By: Antonio Alvau, MSc)

Poster #4
LACK OF MEMBRANE ESTROGEN RECEPTOR ALPHA LEADS TO SPERM ABNORMALITIES, DECREASED SPERM MOTILITY AND VIABILITY, AND PROGRESSIVE INFERTILITY IN MALE MICE
Manjunatha Nanjappa, PhD[1]; Theresa Medrano, MS[1]; Seth Locker[1]; Rex Hess, PhD[2]; Ali Pedram, PhD[3]; Ellis Levin, MD[4] and Paul Cooke, PhD[1]
[1]University of Florida, Gainesville, FL; [2]University of Illinois at Urbana-Champaign, Urbana, IL; [3]University of California, Irvine, CA; [4]University of California, Irvine, CA and Department of Veterans Affairs Medical Center, Long Beach, CA
(Presented By: Manjunatha Ketumaran Nanjappa, BVSc & AH, MVSc, PhD)

Poster #5
LONG-TERM BRDU LABELING IDENTIFIES MITOTICALLY ACTIVE ADARK AND APALE SPERMATOGONIA IN ADULT RHESUS MACAQUE TESTES
Adetunji Fayomi, DVM, MVSc[1]; Brian P. Hermann, PhD[2]; Gary R. Marshall, PhD[3] and Kyle E. Orwig, PhD[4]
[1]Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine; [2]Department of Biology, University of Texas San Antonio; [3]Department of Natural Sciences, Chatham University; [4]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine
(Presented By: Adetunji Fayomi)

Poster #6
PROTEIN TYROSINE KINASE 7 SIGNALS THROUGH MYOSIN II TO REGULATE PLANAR CELL POLARITY-DEPENDENT TUBULAR MORPHOGENESIS OF THE WOLFFIAN/EPIDIDYMAL DUCT, WHICH IN TURN IS IMPORTANT FOR THE ACQUISITION OF SPERM MOTILITY
Bingfang Xu, PhD[1]; Angela M. Washington, BS[1]; Raquel Fantin-Domeniconi, PhD[2]; Ana Cláudia Ferreira-Souza, MS[3]; Xiaowei Lu, PhD[1]; Sutherland Ann, PhD[1] and Barry T. Hinton, PhD[1]
[1]University of Virginia; [2]Institute of Biosciences – UNESP, Botucatu, Brazil; [3]Federal University of Viçosa, Viçosa, Brazil
(Presented By: Bingfang Xu, PhD)

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POSTER SESSION I

Poster #7
ANDROGEN RECEPTOR DEGRADATION BY SULFORAPHANE ENHANCES THE EFFICACY OF ANTI-ANDROGENS AGAINST AGGRESSIVE PROSTATE CANCER CELLS
Namrata Khurana, PhD[1]; Sudha Talwar[1]; Partha Chandra[2]; Asim Abdel-Mageed[1]; Debasis Mondal[2] and Suresh Sikka[1]
[1]Department of Urology, Tulane University; [2]Department of Pharmacology, Tulane University School of Medicine
(Presented By: Namrata Khurana, M.Tech)

Poster #8
CYSTEINE-RICH SECRETORY PROTEIN 3 (CRISP-3) IS A CANDIDATE BIOMARKER OF TESTICULAR DYSFUNCTION IN ADOLESCENTS WITH VARICOCELE
Larissa Berloffa Belardin, BSc; Mariana Camargo, MSc; Paula Intasqui Lopes, MSc; Paula Toni Del Giudice, PhD; Daniel Suslik Zylbersztejn, MD, PhD; Ricardo Pimenta Bertolla, DVM, PhD and Agnaldo Pereira Cedenho, MD, PhD
Department of Surgery, Division of Urology, Universidade Federal de São Paulo
(Presented By: Larissa Berloffa Belardin, BSc)

Poster #9
IFT140 IS A NOVEL CANDIDATE GENE FOR IMPAIRED SPERMATOGENESIS: IDENTIFICATION BY WHOLE EXOME SEQUENCING AND VALIDATION WITH SANGER SEQUENCING
Amin Herati, MD; Peter Butler, BS; Cenk Cengiz, BS; Matthew Bainbridge, PhD; James Lupski, MD; PhD, Richard Gibbs, PhD; Shalini Jhangiani, MS; Larry Lipshultz, MD and Dolores Lamb, PhD
Baylor College of Medicine
(Presented By: Amin S. Herati, MD)

Poster #10
CASANOVA: A MULTICLASS SUPPORT VECTOR MACHINE MODEL FOR THE CLASSIFICATION OF HUMAN SPERM MOTILITY PATTERNS
Summer Goodson, PhD[1]; Sarah White[2]; Alicia Stevans[3]; Sanjana Bhat[3]; Chia-Yu Kao[2]; Scott Jaworski[1]; Tamara Marlowe[1]; Martin Kohlmeier, MD, PhD[1]; Leonard McMillan, PhD[2]; Steven Zeisel, MD, PhD[1] and Deborah O'Brien, PhD[3]
[1]University of North Carolina at Chapel Hill Nutrition Research Institute; [2]Department of Computer Science, University of North Carolina at Chapel Hill; [3]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill
(Presented By: Summer Goodson, PhD)

Poster #11
THE EFFECT OF ORCHIECTOMY ON FUNCTIONAL ASPECTS OF SPERM AND OXIDATIVE STRESS OF SEMINAL PLASMA FROM PATIENTS WITH TESTICULAR TUMORS
Maria Beatriz Ribeiro de Andrade, Paula Intasqui, Larissa Belardin, Mariana Antoniassi, Danielle Tibaldi, Ricardo Pimenta Bertolla and Deborah Spaine
Sao Paulo Federal University
(Presented By: Maria Beatriz Ribeiro De Andrade, BSc)

Poster #12
CONSISTENT DIFFERENCES AMONG MEN IN CAPACITATION TIMING COULD PERSONALIZE IUI/ART
AJ Travis, VMD, PhD[1]; C Cardona, PhD[2]; MA Moody[2]; AJ Simpson[2]; EK Seaman, MD[3] and GC Ostermeier, PhD[2]
[1]Cornell University, Androvia LifeSciences; [2]Androvia LifeSciences; [3]Urology Group of New Jersey
(Presented By: Alexander J. Travis, VMD, PhD)

Poster #13
ASSOCIATION BETWEEN TESTOSTERONE, SEX HORMONE-BINDING GLOBULIN, AND NONALCOHOLIC FATTY LIVER DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS
Sikarin Upala, MD[1] and Anawin Sanguankeo, MD[2]
[1]Bassett Medical Center; [2]Resident physician Bassett Medical Center, Cooperstown, NY
(Presented By: Sikarin Upala, MD)

Poster #14
REGULATION OF SEMINIFEROUS TUBULE-ASSOCIATED STEM LEYDIG CELLS IN ADULT RAT TESTES
Xiaoheng Li[1]; Zhao Wang, PhD[2]; Renshan Ge, PhD[1]; Barry Zirkin, PhD[3] and Haolin Chen, PhD[3]
[1]Wenzhou Medical University, China; [2]Jiamusi University, Jiamusi, China; [3]Johns Hopkins Bloomberg SPH
(Presented By: Xiao-Heng Li, MS)

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POSTER SESSION I

Poster #15
ZINC TRANSPORTERS IN MALE GERM CELLS ARE AFFECTED BY TREATMENT OF MALE RATS WITH CYCLOPHOSPHAMIDE (CPA)
Anne Marie Downey, BSc[1]; Barbara F. Hales, PhD[1] and Bernard Robaire, PhD[2]
[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada; [2]Departments of Pharmacology and Therapeutics and of Obstetrics and Gynecology, McGill University, Montreal, Canada
(Presented By: Anne Marie Downey, BSc)

Poster #16
SMOKING ALTERS SPERM FUNCTION AND THE PROTEOME PROFILE IN MEN WITH VARICOCELE
Mariana Antoniassi, BSc, MSc[1]; Mariana Camargo, BSc, MSc[1]; Paula Intasqui, BSc, MSc[1]; Karina Cardozo, PhD[2]; Valdemir Carvalho, PhD[2] and Ricardo Bertolla, DVM, PhD[1]
[1]Department of Surgery, Division of Urology, Human Reproduction Section, Sao Paulo Federal University; [2]Fleury Group
(Presented By: Mariana Pereira Antoniassi, BSc, MSc)

Poster #17
PATERNAL LIFESTYLE FACTORS ASSOCIATED WITH SPERM DNA DAMAGE
Surabhi Gautam, PhD; Scholar[1]; Shiv Basant Kumar, MSc[1]; Shilpa Bisht, PhD, Scholar[1]; Bhavna Chawla, Associate Professor[2] and Rima Dada, Professor[1]
[1]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; [2]Retinoblastoma Clinic, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Surabhi Gautam, PhD Scholar)

Poster #18
STIMULATORY EFFECTS OF COMBINED ENDOCRINE DISRUPTORS ON MA-10 LEYDIG CELL STEROID PRODUCTION AND LIPID HOMEOSTASIS
Steven Jones, MSc[1]; Annie Boisverts, MSc[1]; Andrada Naghi, BSc[1]; Francoise Hullin-Matsuda, PhD[2]; Peter Greimel, PhD[3]; Toshihide Kobayashi, PhD[3]; Vassilios Papadopoulos, DPharm, PhD[1] and Martine Culty, PhD[1]
[1]McGill University; [2]INSERM UMR1060; [3]RIKEN Institute
(Presented By: Steven Jones, BSc, MSc)

Poster #19
IN UTERO EXPOSURE TO DEHP ALTERS STEROIDOGENESIS IN THE ADULT MALE OFFSPRING: REVERSIBILITY OF THE EFFECTS USING PHARMACOLOGICAL MEANS
Sunghoon Lee, BSc[1]; Daniel B. Martinez-Arguelles, MD, PhD[2] and Vassilios Papadopoulos, DPharm, PhD[3]
[1]The Research Institute of the McGill University Health Centre, McGill University Department of Biochemistry; [2]The Research Institute of the McGill University Health Centre, McGill University Department of Medicine; [3]The Research Institute of the McGill University Health Centre, McGill University Department of Biochemistry, McGill University Department of Medicine
(Presented By: Sunghoon Lee, BSc, MSc)

Poster #20
PATERNAL STRESS REPROGRAMS EPIGENETIC MARKS IN SPERM AND EPIDIDYMIS: IMPLICATIONS FOR LONG-TERM TRANSMISSION OF EXPERIENCE
Jennifer Chan and Tracy Bale, PhD
University of Pennsylvania
(Presented By: Jennifer C. Chan, BA)

Poster #21
EFFECT OF PRENATAL EXPOSURE TO 1,2-CYCLOHEXANE DICARBOXYLIC ACID DIISONONYL ESTER (DINCH) ON OFFSPRING TESTICULAR FUNCTION
Enrico Campioli, PharmD, PhD[1]; Matthew Lau[2]; Sunghoon Lee, BSc[3] and Vassilios Papadopoulos, PharmD, PhD[4]
[1]RI-MUHC, Department of Medicine; [2]RI-MUHC, Department of Pharmacology & Therapeutics; [3]RI-MUHC, Department of Biochemistry; [4]RI-MUHC, Departments of Medicine, Biochemistry and Pharmacology & Therapeutics
(Presented By: Enrico Campioli, PharmD, PhD)

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POSTER SESSION I

Poster #22

NEGATIVE EFFECTS OF MARIJUANA (CANNABIS SATIVA) CONSUMPTION IN MALE FERTILITY POTENTIAL: TESTIS VOLUME, SEXUAL HORMONE LEVELS AND SEMINAL PARAMETERS EVALUATION

Juliana R. Pariz, BSc, MSc[123]; Rosa Alice C. Monteiro, BSc[3]; Paulo Hilário Saldiva, MD, PhD[2] and Jorge Hallak, MD, PhD[123]
[1]Sector of Andrology, Department of Urology, Universidade de São Paulo, Brazil; [2]Unit of Reproductive Toxicology, Department of Pathology, Universidade de São Paulo, Brazil; [3]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil.
(Presented By: Juliana Risso Pariz, BSc, MSc)

Poster #23

BODY MASS INDEX OR BODY FAT PERCENTAGE? AN INITIAL REPRODUCTIVE OVERVIEW FOR TWO OBESITY CLASSIFICATIONS

Caroline Ranéa, BSc student[123]; Juliana R. Pariz, BSc, MSc[124]; Rosa Alice Monteiro, BSc[2]; Inari Ciccone, BSc[2]; Elaine Maria F. Costa, MD, PhD[2] and Jorge Hallak, MD, PhD[1],[2,4]
[1]Sector of Andrology, Department of Urology, Universidade de São Paulo, Brazil; [2]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil; [3]Universidade de Santo Amaro, Brazil.; [4]Unit of Reproductive Toxicology, Department of Pathology, Universidade de São Paulo, Brazil.
(Presented By: Caroline Ranéa)

Poster #24

A NOVEL STEM LEYDIG CELL CULTURE SYSTEM AND ITS USE IN ASSESSING THE EFFECTS OF PHTHALATE EXPOSURE ON LEYDIG CELL DEVELOPMENT

Rose Schrott, BA; Barry Zirkin, PhD and Haolin Chen, PhD
Johns Hopkins School of Public Health
(Presented By: Rose Schrott, BA)

Poster #25

DRUG TOXICITY EFFECTS ON HUMAN TESTIS FUNCTIONS: 2D VERSUS 3D IN VITRO CULTURE

Samuel Pendergraft, MS[1]; Hooman Sadri-Ardekani, MD, PhD[2]; Anthony Atala, MD[2] and Colin Bishop, PhD[1]
[1]Wake Forest Institute for Regenerative Medicine; [2]Wake Forest Institute for Regenerative Medicine, Wake Forest Baptist Medical Center Department of Urology
(Presented By: Samuel Pendergraft, MS)

Poster #26

PHTHALATE EXPOSURE AND SPERM DNA METHYLATION AMONG MEN SEEKING IVF TREATMENT

Haotian Wu[1]; Cynthia Sites, MD[2]; Rahil Tayyab, PhD[2] and J. Richard Pilsner, PhD[1]
[1]UMass Amherst School of Public Health and Health Sciences, Department of Environmental Health Sciences; [2]Baystate Hospital, Department of Obstetrics and Gynecology
(Presented By: Haotian Wu)

Poster #27

THE BINDER OF SPERM HOMOLOG 2 (BSPH2) GENE KNOCKOUT BY CRISPR/CAS9 SYSTEM AND THE EFFECT ON IN VIVO FERTILITY

Marzieh Eskandari-Shahraki, PhD[1]; Bruno Prud'homme, Research Assistance[2] and Puttaswamy Manjunath, Professor[3]
[1]University of Montreal; [2]Hôpital Maisonneuve-Rosemont; [3]University of Montral
(Presented By: Marzieh Eskandari Shahraki, PhD student)

Poster #28

ANDROGENS ARE ESSENTIAL FOR EPITHELIAL CELL RECOVERY AFTER EFFERENT DUCT LIGATION (EDL) IN THE PRE-PUBERTAL EPIDIDYMIS

Bongki Kim, PhD and Sylvie Breton, PhD
Massachusetts General Hospital
(Presented By: Bongki Kim, PhD)

Poster #29

REGULATION OF LUMINAL ACIDIFICATION BY PRINCIPAL AND CLEAR CELLS IN THE MOUSE EPIDIDYMIS

Yoo-Jin Park, PhD; Bongki Kim, PhD and Sylvie Breton, PhD
Massachusetts General Hospital, Harvard Medical School
(Presented By: Yoojin Park, PhD)

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POSTER SESSION I

Poster #30
ROLE OF DICER1-DEPENDENT MICRORNAS IN THE PARACRINE CONTROL OF EPIDIDYMAL GENE EXPRESSION
Olivia Jerczynski Master[1]; Nicolas Lacroix-Pépin, PhD[1]; Eric Boilard, PhD[1]; Ézequiel Calvo, PhD[1]; Michel A. Fortier, PhD[1]; Ida Björkgren, PhD[2]; Petra Sipilä, PhD[2] and Clémence Belleannée, PhD[1]
[1]Laval University; [2]University of Turku
(Presented By: Olivia Jerczynski)

Poster #31
INTERLEUKIN 6 INHIBITS THE DIFFERENTIATION OF RAT STEM LEYDIG CELLS INTO LEYDIG CELL LINEAGE
Yiyan Wang, MD; Quifan Wang, PhD; Guimin Wang, PhD; Xiaoheng Li, MS; Linxi Li, PhD; Yanfang Bai, MD; Baiping Mao, MD; Haolin Chen, PhD; Jingjing Guo, PhD and Renshan Ge, PhD
Center of Scientific Research, The 2nd Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang
(Presented By: Yiyan Wang)

Poster #32
EFFECTS OF IN UTERO EXPOSURE OF TETRABUTYLTIN ON FETAL LEYDIG CELLS
Yaoyao Dong, MD; Haitao Yang, PhD; Qiufan Wang, PhD; Yiyan Wang, MD; Xiaoheng Li, MD; Xiaomin Chen, PhD; Qingquan Lian, PhD and Ren-Shan Ge, MD
The Second Affiliated Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, PR China
(Presented By: Yaoyao Dong, Sr., master)

Poster #33
EFFECT OF PROPOFOL ON THE STEROIDOGENESIS OF RAT IMMATURE LEYDIG CELLS
Xiaolong Wu; Hongguo Guan, MD; Senlin Li, MD; Lili Li Li, MD; Songyi Zhou, MD; Qiuxia Shen, MD; Xiaojun Li, MD; Xiaojun Lian, PhD and Ren-Shan Ge, MD
The Second Affiliated Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, PR China
(Presented By: Xiaolong Wu, Sr., master)

Poster #34
IMMATURE SERTOLI CELLS HAS HIGHER EXPRESSION OF 17?-HYDROXYSTEROID DEHYDROGENASE 3 THAN FETAL LEYDIG CELLS IN NEONATAL RAT TESTIS
Baiping Mao, MD; Linxi Li, PhD; Chao Li, PhD; Xiudi Wang, Bachelor degree; Ying Wu, MD; Yiyan Wang, MD; Xiaoheng Li, MD; Xiaomin Chen, PhD and Ren-Shan Ge, MD
The Second Affiliated Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, PR China
(Presented By: Baiping Mao, Sr., master's degree)

Poster #35
ANDROGEN-RECEPTOR EXPRESSION AND LOCALIZATION IN MUSSEL SPERM
Jeremy Feng and Wei Zhu, PhD
Neuroscience Institute
(Presented By: Jeremy Feng)

Poster #36 - WITHDRAWN

Poster #37
CHES-1-LIKE, THE ORTHOLOG OF A NON-OBSTRUCTIVE AZOOSPERMIA ASSOCIATED GENE, BLOCKS GERMLINE STEM CELL DIFFERENTIATION THROUGH UPREGULATING DPP EXPRESSION IN THE DROSOPHILA TESTIS.
Jun Yu, PhD[1]; Yu-Juan Liu, bachelor[1]; Xiang Lan, PhD[2]; Hao Wu, PhD[1]; Jia-Hao Sha, PhD[1] and Chao Tong, PhD[2]
[1]State Key Laboratory of Reproductive Medicine, Department of Histology and Embryology, Nanjing Medical University; [2]Life Sciences Institute and Innovation Center for Cell Signaling Network, Zhejiang University
(Presented By: Jun Yu, PhD)

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POSTER SESSION I

Poster #38
LEUKEMIA INHIBITORY FACTOR STIMULATES STEROIDOGENESIS OF RAT IMMATURE LEYDIG CELLS VIA INCREASING THE EXPRESSION OF STEROIDOGENIC ACUTE REGULATORY PROTEIN
Xiaoheng Li, MD; Guimin Wang, MD; Chao Li, PhD; Kaimin Yuan, PhD; Zhijian Su, MD; Ying Su, MD and Ren-shan Ge, MD
The Second Affiliated Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, PR China
(Presented By: Xiao-Heng Li, MS)

Poster #39
CALCIUM IONOPHORE A23187 INDUCES IN-VITRO FERTILIZATION CAPACITY IN SPERM FROM STERILE KNOCKOUT MODELS
Felipe Navarrete, BS and Pablo Visconti, PhD
University of Massachusetts Amherst
(Presented By: Felipe A. Navarrete, BS)

Poster #40
ALTERNATIVE SEX HORMONE-BINDING GLOBULIN PROMOTER TRANSCRIPTIONAL ACTIVITY IN TESTICULAR GERM CELLS
Warren Meyers, BSc and Geoffrey Hammond, PhD
Departments of Obstetrics and Gynaecology & Cellular and Physiological Sciences, University of British Columbia
(Presented By: Warren Meyers, BSc)

Poster #41
ROLE OF PATERNAL FACTORS IN EARLY EMBRYONIC DEVELOPMENT
Manoj Kumar, PhD[1]; Vidhu Dhawan, MD[1]; Dipika Deka, MD[2]; Vatsala Dadhwal, MD[2] and Rima Dada, PhD[1]
[1]Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; [2]Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Manoj Kumar, MSc, PhD)

Poster #42
MICRORNA EXPRESSION FOLLOWING LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION OF RAT TESTIS
Mitchell Parker and Michael Palladino, PhD
Monmouth University
(Presented By: Mitchell Isaac Parker)

Poster #43
MAPPING GENETIC HETEROGENEITY OF VIABLE AND NON-VIABLE SPERM
Liina Nagirnaja, PhD[1]; Michiel Noordam, PhD[2] and Donald Conrad, PhD[3]
[1]Department of Genetics, Washington University in St. Louis; [2]Maastricht University, Netherlands; [3]Department of Genetics, Washington University School of Medicine
(Presented By: Liina Nagirnaja, PhD)

Poster #44
HIGH DOSE FOLIC ACID SUPPLEMENTATION AND METHYLENETETRAHYDROFOLATE REDUCTASE HAPLOINSUFFICIENCY RESULT IN HYPOMETHYLATION OF THE SPERM DNA METHYLOME IN MICE
Mahmoud Aarabi, MD, PhD[1]; Donovan Chan, PhD[2]; Mylene Landry, MSc[2]; Lundi Ly, MSc[1]; Karen Christensen, PhD[2]; Qing Wu, MSc[2]; Rima Rozen, PhD[1] and Jacquetta Trasler, MD, PhD[3]
[1]Department of Human Genetics, McGill University, and Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, QC; [2]Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, QC; [3]Departments of Pediatrics, Human Genetics, Pharmacology & Therapeutics, McGill University, and Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, QC
(Presented By: Mahmoud Aarabi, MD, PhD)

Poster #45 - WITHDRAWN

Poster #46
HYPOASTHENOZOOSPERMIA CORRELATES WITH INCREASED DNA SPERM FRAGMENTATION
Marcela Lozano Mendoza; Hector Godoy, MD and José Manuel Lozano Sánchez, MD
(Presented By: Marcela Lozano Mendoza)

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POSTER SESSION I

Poster #47
SEMEN OXIDATIVE REDUCTION POTENTIAL (ORP) IS RELATED TO ABNORMAL SEMEN PARAMETERS IN MALE FACTOR INFERTILITY
Jaideep Toor, PhD; Lucelia Daniels; Faysal Yafi; Wayne Hellstrom and Suresh Sikka
Department of Urology, Tulane University
(Presented By: Jaideep Singh Toor, PhD)

Poster #48
CAN CAFFEINE AND MELATONIN IMPROVE THE QUALITY OF FROZEN-THAWED HUMAN SPERM?
Juliana R. Pariz, BSc, MSc[123] and Jorge Hallak, MD, PhD[123]
[1]Sector of Andrology, Department of Urology, Universidade de São Paulo, Brazil; [2]Unit of Reproductive Toxicology, Department of Pathology, Universidade de São Paulo, Brazil; [3]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil.
(Presented By: Juliana Risso Pariz, BSc, MSc)

Poster #49
MELANOMA ANTIGEN PROTEIN MAGEC1 MUTATION IDENTIFIED IN FAMILIAL NON-OBSTRUCTIVE AZOOSPERMIA
Alexander W. Pastuszak, MD, PhD; Cenk Cengiz, BS; Mir Reza Bekheirnia, MD; Larry I. Lipshultz, MD and Dolores J. Lamb, PhD
Baylor College of Medicine
(Presented By: Alexander W. Pastuszak, MD, PhD)

Poster #50
ELECTROEJACULATION PERFORMED IN PATIENTS WITH SPINAL CORD INJURIES – A SINGLE CENTER 21 YEARS EXPERIENCE
Timo Soeterik, BSc[1]; Paul Veenboer, PhD, MD[1]; Ralph Oude Ophuis, PhD, MSc[2] and Tycho Lock, MD, FEBU[1]
[1]Department of Urology, University Medical Center Utrecht; [2]Department of Reproductive Medicine, University Medical Center Utrecht
(Presented By: Timo Soeterik, BSc)

Poster #51
SEMINAL OXIDATIVE STRESS, DNA DAMAGE AND TELOMERE DYNAMICS: ROLE IN IDIOPATHIC MALE INFERTILITY
Swetasmita Mishra, MSc[1]; Rajeev Kumar, MD[2]; Neena Malhotra, MD[3] and Neeta Singh, MD[3]
[1]Lab for Molecular Reproduction and Genetics; [2]Department of Urology, All India Institute of Medical Sciences; [3]Department of Obstetrics & Gynaecology, All India Institute of Medical Sciences
(Presented By: Swetasmita Mishra, MSc)

Poster #52
GPR56-NULL MALE MICE ARE SUBFERTILE AND HAVE DEFECTIVE SPERMATOGENESIS, DECAPITATED SPERM, REDUCED MOTILITY, AND PROVIDE EVIDENCE FOR THE EXISTENCE OF A SPERM QUALITY CONTROL MECHANISM IN THE EPIDIDYMIS
Laura Ellis; Brennan Terhune-Cotter; Amy Northrop, BS and James Foster, PhD
Randolph-Macon College
(Presented By: Laura Peyton Ellis)

Poster #53
PRIOR FERTILITY-SPECIFIC HEALTHCARE UTILIZATION OF COUPLES SEEKING UROLOGICAL EVALUATION FOR MALE INFERTILITY
Christopher Gaffney, BA; Matthew Pagano, MD and Peter Stahl, MD
Columbia
(Presented By: Christopher Daniel Gaffney, BA)

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POSTER SESSION I

Poster #54
IS THE AREA OF THE GENITAL CORTICAL REPRESENTATION ASSOCIATED WITH COPULATORY BEHAVIOR PERFORMANCE IN MALE RATS?
Diana Esquivel-Franco, Graduate Student[1]; Alonso Fernández-Guasti, PhD[2]; Luisa Jiménez-Sánchez, Bachelor of Science[1]; Jesús Ramírez-Santos, PhD[1] and Gabriel Gutiérrez-Ospina, MD, PhD[1]
[1]Dept. of Cell Biology and Physiology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México; [2]Dept. of Pharmacobiology, Centro de Investigaciones y Estudios Avanzados, Instituto Politécnico Nacional
(Presented By: Diana Carolina Esquivel Franco, BS)

Poster #56
EVALUATION OF ELECTROMAGNETIC FIELD EFFECT ON OXIDATIVE STRESS LEVEL, TESTOSTERONE HORMONE AND SEXUAL BEHAVIOR IN MALE RAT TREATED WITH OMEGA 3
Nava Ainehchi, PhD[1]; Arash Khaki, DVM, PhD[1] and Afshin Zahedi, DVM, PhD[2]
[1]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences; [2]Department of Pathology, Rasht Branch, Islamic Azad university, Rasht, Iran
(Presented By: Nava Ainehchi, PhD)

Poster #57
UNDERLYING SEXUAL DYSFUNCTION IN MEN PRESENTING WITH VARICOCELE
Matthew Pagano, MD; Alison Levy, MD; Christopher Gaffney, BA; Ifeanyi Onyeji, BA and Peter Stahl, MD
Columbia University Medical Center
(Presented By: Matthew J. Pagano, MD)

Poster #58
OUTCOMES OF INFLATABLE PENILE PROSTHESIS SURGERY: AN ANALYSIS OF A NEW YORK STATE DATABASE
Ifeanyi Onyeji, BA[1]; Wilson Sui, BA[1]; Matthew Pagano, MD[1]; Aaron Weinberg, MD[1]; Maxwell James, BS[1]; Marissa Velez, MD[1]; Doron Stember, MD[2] and Peter Stahl, MD[1]
[1]Columbia University Medical Center, Department of Urology; [2]Mount Sinai Ichan School of Medicine, Department of Urology
(Presented By: Ifeanyi Cliff Onyeji, BA)

Poster #59
FOUR-RING CIRCUS: A CASE OF PENILE INCARCERATION
Graham Machen, MD; Tillman Hudson, MD and Jeffery Waxman, MD
Baylor Scott & White Health/the Texas A&M Health Science Center College of Medicine
(Presented By: Graham Luke Machen, MD)

Poster #60
CLAUDINS ARE ALTERED DURING GONOCYTE TO SPERMATOGONIA TRANSITION AND IN SEMINOMAS
Gurpreet Manku[1], Aurelie Hueso[2], Fadi Brimo[3], Peter Chan[4], Pilar Gonzalez-Peramato[5], Nada Jabado[6], Tenzin Hayden[6], Mathieu Bourgey[7], Yasser Riazalhosseini[8] and Martine Culty[1]
[1]The Research Institute of the McGill University Health Centre, and the Departments of Medicine and Pharmacology & Therapeutics at McGill University; [2]The Research Institute of the McGill University Health Centre and the Department of Medicine at McGill University; [3]The Research Institute of the McGill University Health Centre and the Department of Pathology at McGill University; [4]The Research Institute of the McGill University Health Centre and the Department of Surgery at McGill University; [5]Department of Pathology, La Paz University Hospital, Universidad Autonoma de Madrid, Madrid, Spain; [6]Department of Pediatrics at McGill University; [7]Genome Quebec Innovation Centre; [8]Genome Quebec Innovation Centre and the Department of Human Genetics at McGill University
(Presented By: Gurpreet Manku, BSc, PhD)

Poster #61
HUMAN SPERM NUCLEAR MORPHOMETRY FOR SEMEN QUALITY ASSESSMENT AS MEASURED WITH FOURIER HARMONIC ANALYSIS
Megan Krautkramer, MS and John Parrish, PhD
UW Madison
(Presented By: Megan Krautkramer, MS)

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POSTER SESSION I

Poster #62
COMET DISTRIBUTION PLOT: PROVIDES UNIQUE PATIENT PROFILING ALONG WITH HIGH DIAGNOSTIC VALUE
Luke Simon, PhD; Kenneth Aston, PhD; Benjamin Emery, Masters and Douglas Carrell, PhD
University of Utah
(Presented By: Luke Simon, PhD)

Poster #63
WITHDRAWN

Poster #64
EXPRESSION OF A 52 KDA PEPTIDE FROM MUCIN-5B IN THE SEMINAL PLASMA AS A MEASURE FOR MONITORING SEMINAL OXIDATIVE STRESS
Paula Intasqui, MSc, BSc; Larissa Belardin, BSc; Mariana Camargo, MSc, BSc; Daniel Zylbersztejn, MD, PhD and Ricardo Bertolla, DVM, PhD
Division of Urology, Department of Surgery
(Presented By: Paula Intasqui Lopes, MSc, BSc)

Poster #65
MULTIPLE MECHANISMS OF SPERM ASSOCIATED ANTIGEN 6 (SPAG6)-ASSOCIATED MALE FERTILITY: MOTILITY AND SPERMATOGENESIS
Hong Liu, Wei Li, MD[1]; Ling Zhang, PhD[2]; Zhengang Zhang, MD, PhD[3]; Junpin Liu, MD[4]; Guanglun Pin, MD[5]; Hongfei Li, MD[1]; Maria Teves, PhD[1]; Shizheng Song, MD, PhD[5]; Chunghee Cho, PhD[6]; Uwe Knippschild, PhD[7]; Jerome Strauss, MD, PhD[1] and Zhibing Zhang, MD, PhD[1]
[1]Virginia Commonwealth University; [2]Wuhan University of Science and Technology; [3]Tongji Medical College; [4]Wuhan Hospital for The Prevention and Treatment of Occupational Diseases; [5]Wuhan University of Science and Technology; [6]Gwangju Institute of Science and Technology; [7]the Medical University of Ulm
(Presented By: Hong Liu, Master)

Poster #66
IDENTIFICATION OF THE SOURCE OF CHOLESTEROL USED FOR STEROID BIOSYNTHESIS IN PRIMARY RAT LEYDIG CELLS
Sathvika Venugopal, PhD; Daniel Martinez-Arguelles, MD, PhD and Vassilios Papadopoulos, DPharm, PhD
Research Institute of the McGill University Health Centre and Department of Medicine, McGill University
(Presented By: Sathvika Venugopal)

Poster #67
LOW DIETARY IRON INTAKE DECREASES EPIDIDYMAL SPERM COUNTS IN MICE
Joseph Gabrielsen, MD, PhD[1]; Lipika Salaye[2]; Cigdem Tanrikut, MD[1] and Donald McClain, MD, PhD[2]
[1]Massachusetts General Hospital; [2]Wake Forest Baptist Medical Center
(Presented By: Joseph S. Gabrielsen, MD, PhD)

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POSTER SESSION I

Poster #68
REGULATION OF DISTAL GATA4 1B PROMOTER ACTIVITY
Vanessa Théberge[1], Francis Bergeron[1], Charles Joly-Beauparlant[2], Audrey Lemaçon[2], Arnaud Droit[2], Clémence Belleannée[3] and Robert Viger[3]
[1]Reproduction, Mother and Child Health, Centre de recherche du CHU de Quebec-Université Laval and Centre de recherche en reproduction, développement et santé intergénérationnelle , Quebec City, Canada; [2]Department of Molecular Medicine, Laval University & Centre de recherche du CHU de Quebec-Université Laval, Quebec City, Canada; [3]Reproduction, Mother and Child Health, Centre de recherche du CHU de Quebec-Université Laval and Centre de recherche en reproduction, développement et santé intergénérationnelle , Quebec City, Canada . Department of Obstetrics, Gynecology, and Reproduction, Faculty of Medicine, Laval University, Quebec City, Canada
(Presented By: Vanessa Theberge)

Poster #69
MULTIPLE COMPONENTS OF THE MEIOSIS-ACTIVATING STEROL SIGNALLING AND TRANSCRIPTION FACTOR NETWORKS DIFFERENTIALLY REGULATE SERTOLI CELL GENE EXPRESSION
KeumSil Hwang, BS, MS[1]; Lyann Mitchell, BS[1] and Patricia Morris, BS, MS, PhD[2]
[1]Population Council; [2]Population Council and The Rockefeller University
(Presented By: Keumsil Hwang, BS, MS)

Poster #70
NA/K-ATPASE REGULATES EXPRESSION OF CONNEXIN 43 AND CLAUDIN 11 IN RAT PREPUBERTAL SERTOLI CELLS
Gayathri Rajamanickam, BVSc[1] and Jacob Thundathil, DVM, MVSc, PhD[2]
[1]Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary; [2]Associate Professor, Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary
(Presented By: Gayathri Rajamanickam, BVSc)

Poster #71
TRANSCRIPTION FACTORS SF-1 AND CJUN COOPERATE TO ACTIVATE THE FDX1 PROMOTER IN MA-10 LEYDIG CELLS
Pauline Roumaud, MSc; Arlette Rwigemera, MSc and Luc J. Martin, PhD
Biology Department, Université de Moncton, Moncton, New-Brunswick, Canada, E1A 3E9
(Presented By: Pauline Roumaud, MSc)

Poster #72
TRANSDIFFERENTIATION OF RAT STEM LEYDIG CELLS INTO PROSTATIC AND UTERINE EPITHELIUM, BUT NOT EPIDERMIS
Manjunatha Nanjappa, PhD[1]; Theresa Medrano, MS[1]; Haolin Chen, PhD[2]; Barry Zirkin, PhD[2] and Paul Cooke, PhD[1]
[1]University of Florida, Gainesville, FL; [2]Johns Hopkins University, Baltimore, MD
(Presented By: Manjunatha Ketumaran Nanjappa, BVSc & AH, MVSc, PhD)

Poster #73
TRANSLATIONAL DYNAMICS OF GERM CELL DEVELOPMENT
Ana Cristina Lima[123], Nicholas Ho Rui Yuan[3], António Amorim[245], Donald Conrad[36] and Alexandra Lopes[25]
[1]Abel Salazar Institute of Biomedical Sciences, University of Porto, Portugal; [2]Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal; [3]Dep. of Genetics, Washington University School of Medicine, St. Louis, USA; [4]Faculty of Sciences of the University of Porto, Portugal; [5]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal – I3S; [6]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA
(Presented By: Ana Cristina Lima, MSc)

Poster #74
THE JAG1/NOTCH CANONICAL EFFECTORS HES1 AND HEY1 BIND TO THE GDNF PROXIMAL PROMOTER TO REPRESS ITS TRANSCRIPTION IN SERTOLI CELLS
Parag Parekh, PhD[1]; Thomas Garcia, PhD[2]; Krishna Sinha, PhD[3] and Marie-Claude Hofmann, PhD[1]
[1]UT MD Anderson Cancer Center, Houston, TX; [2]UT MD Anderson Cancer Center and University of Houston Clear Lake, Houston, TX; [3]UT MD Anderson Cancer Center and University of Texas Health Sciences Center, Houston, TX
(Presented By: Parag Parekh, PhD)

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POSTER SESSION I

Poster #75
GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) PROMOTES SPERMATOGENIC REGENERATION AFTER HIGH-DOSE ALKYLATING CHEMOTHERAPY.
Travis Kotzur; Roberto Benavides-Garcia, BS; Jennifer Mecklenburg, MS and Brian Hermann, PhD
University of Texas at San Antonio
(Presented By: Travis Kotzur)

Poster #76
EVALUATION OF THE EFFECT OF TWO DIFFERENT CULTURE CONDITIONS ON THE IN VITRO GROWTH OF CD90+VE HUMAN TESTICULAR CELLS USING NEXT GENERATION SEQUENCING (NGS)
Itai Gat, MD[1]; Melissa Felice[1]; Leila Maghen[1]; Ekaterina Shlush[1]; Khaled Zohni[1]; Keith Jarvi[2]; Kirk Lo[2]; Andree Gauthier Fisher[1] and Clifford Librach[1]
[1]CReATe Fertility Centre, Toronto, Ontario, Canada; [2]Urology Deprtment, Mount Sinai Hospital, Toronto, Ontario, Canada
(Presented By: Itai Gat, MD)

Poster #77
REPEATED DIFFERENTIAL PLATING RESULTED IN REDUCED MURINE TESTICULAR GERM CELL PROLIFERATION AND COLONY FORMATION
Itai Gat, MD; Leila Maghen; Ekaterina Shlush; Shlomit Kenigsberg; Khaled Zohni; Melissa Filice; Andrée Gauthier-Fisher and Clifford Librach
CReATe Fertility Centre, Toronto, Ontario, Canada
(Presented By: Itai Gat, MD)

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POSTER SESSION II

Monday, April 4, 2016
***Poster Session II**
11:15 a.m. – 12:30 p.m.
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

Poster #55
IMPACT OF NUMBER OF CYCLES OF COLLAGENASE CLOSTRIDIUM HISTOLYTICUM ON OUTCOMES IN PATIENTS WITH PEYRONIE'S DISEASE
James Anaissie, Faysal A. Yafi, MD, FRCSC; Elizabeth J. Traore; Suresh C. Sikka, PhD, HCLD and Wayne J.G. Hellstrom, MD, FACS
Tulane University
(Presented By: James Anaissie)

Poster #78
SUBTLE BLOOD LOSS CAN BE ASSOCIATED WITH A LACK OF RESPONSE TO TESTOSTERONE REPLACEMENT THERAPY
Phuong Tran, MD; Robert Tan, MD and Patrick Whitfield, NP-C
Low T Institute
(Presented By: Phuong Tran, MD)

Poster #79
MECHANISM OF HYPOGONADISM IN THE TRANSGENIC SICKLE CELL MOUSE
Arthur Burnett, MD; Haolin Chen, PhD; Yuxi Zhang, MD; Terry Brown, PhD; Barry Zirkin, PhD and Biljana Musicki, PhD
Johns Hopkins Hospital
(Presented By: Arthur L. Burnett, II, MD, MBA)

Poster #80
IMPROVING FERTILITY OF BULLS AND BUFFALO-BULLS
Gamal Elamrawi, PhD
Aleaxndria University/Faculty of Veterinary Medicine
(Presented By: Gamal Elamrawi, PhD)

Poster #81
THE INNOVATIVE COMBINATION OF THE HERBAL REMEDY IN INFERTILE COUPLES WITH MALE FACTOR COMPARATIVE SUCCESS OF ASSISTED REPRODUCTIVE TECHNIQUES
Arash Khaki, PhD[1]; Elaheh Uladsaheb Madarek, PhD[2] and Nava Ainehchi, PhD[2]
[1]Department of Pathobiology,Tabriz Branch,Islamic Azad University,Tabriz,Iran.; [2]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
(Presented By: Arash Khaki, DVM, PhD)

Poster #82
A ROLE OF KIT RECEPTOR SIGNALING FOR PROLIFERATION AND DIFFERENTIATION OF RAT STEM LEYDIG CELLS
Xiaomin Chen, PhD; Shiwen Liu, PhD; Yiyan Wang, PhD; Linxi Li, PhD; Guiming Wang, PhD; Xiaoheng Li, Master; Haolin Chen, PhD; Jingjing Guo, PhD; Han Lin, PhD; Qingquan Lian, PhD and Renshan Ge, PhD
(Presented By: Xiaomin Chen, PhD)

Poster #83
LONGTERM-TREATMENT OF OVERWEIGHT OR OBESE HYPOGONADAL MEN USING INTRAMUSCULAR TESTOSTERONE UNDECANOATE: LONGTERM-EXPERIENCE OF 4-7 YEARS
Michael Zitzmann, MD, PhD[1] and Farid Saad, MD[2]
[1]University Clinics Muenster; [2]Bayer Inc
(Presented By: Michael Zitzmann, MD, PhD)

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POSTER SESSION II

Poster #84
CONTEMPORARY TESTOSTERONE PRESCRIBING PATTERNS AT AN ACADEMIC HEALTH CENTER
Jason Jameson, MD
Mayo Clinic Arizona
(Presented By: Jason J. Jameson, MD)

Poster #85
EFFECT OF LPCN 1021 (ORAL TESTOSTERONE) IN HYPOGONADAL MEN REPORTING PSYCHOSOCIAL SYMPTOMS AT BASELINE – SUBGROUP ANALYSIS OF SOAR (STUDY OF ANDROGEN REPLACEMENT) TRIAL
Martin Miner, MD[1]; Christina Wang, MD[2]; Jed Kaminetsky, MD[3]; Adrian Dobs, MD, MHS[4]; Irwin Goldstein, MD[5]; Anthony Delconte, MD[6]; Nachiappan Chidambaram, PhD[7]; Satish Nachaegari, MS[7]; Mahesh Patel, PhD[7] and Mohit Khera, MD[8]
[1]Brown University and Miriam Hospital; [2]Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA; [3]University Urology Associates, New York, NY; [4]Johns Hopkins University School of Medicine, Baltimore, MD; [5]Alvarado Hospital, San Diego, CA; [6]Lipocine, Inc. Salt Lake City, UT, and Saint Joseph's University, Philadelphia, PA; [7]Lipocine, Inc. Salt Lake Cit, UT; [8]Baylor College of Medicine, Houston, TX
(Presented By: Martin Miner, MD)

Poster #86
NOVEL MOUSE SEMINIFEROUS TUBULE CULTURE SYSTEM TO INVESTIGATE THE INTERACTION BETWEEN THE STEM LEYDIG CELL AND ITS NICHE
Ren-Shan Ge, MD; Guimin Wang, MD; Linxi Li, PhD; Siwen Liu, MD; Yiyan Wang, MD; Jingjing Guo, MD; Haolin Chen, PhD and Qingquan Lian, PhD
The Second Affiliated Hospital of Wenzhou Medical University
(Presented By: Renshan Ge, MD, MS)

Poster #87
RAT SPERM MRNA TRANSCRIPT LEVELS ARE SENSITIVE INDICATORS OF TESTICULAR DYSFUNCTION
Edward Dere, PhD[1]; Aimee Altemus, BS[2]; James Smith, PhD[2]; Jonathan Phillips, PhD[2]; Kerry Blanchard, PhD[2] and Kim Boekelheide, PhD[1]
[1]Brown University; [2]Boehringer Ingelheim Pharmaceuticals, Inc
(Presented By: Edward Dere, PhD)

Poster #88
EXPOSURE TO POLYBROMINATED BIPHENYLS AND SEMEN PARAMETERS, FERTILITY AND ERECTILE FUNCTION IN MEN
Tamar Wainstock, PhD[1]; Steven M. Schrader, PhD[2]; Metrecia L. Terrell, MSPH[1]; Dana Barr, PhD[1]; M. Elizabeth Marder, PhD[1]; George Steinhardt, MD[3]; John J. De Caro, MD[4]; James S. Kesner, PhD[2]; Terry W. Turner, BS[2] and Michele Marcus, MPH, PhD[1]
[1]Emory University; [2]CDC/NIOSH; [3]Helen DeVos Children's Hospital; [4]The Southeast Permanent Medical Group
(Presented By: Steven M. Schrader, PhD)

Poster #89
INFLUENCE OF AIR POLLUTION OF VEHICULAR ORIGIN IN SEMINAL PARAMETERS OF TRAFFIC CONTROLLERS
Jorge Hallak, MD, PhD[123]; Juliana Andrietta, BSc, MSc[14]; Patricia de C. Pieri, BSc, MSc, PhD[3]; Juliana R. Pariz, BSc, MSc[123] and Paulo Hilário Saldiva, MD, PhD[4]
[1]Sector of Andrology, Department of Urology, Universidade de São Paulo, Brazil; [2]Unit of Reproductive Toxicology, Department of Pathology, Universidade de São Paulo, Brazil; [3]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil.; [4]Unit of Reproductive Toxicology, Department of Pathology, Universidade de São Paulo, Brazil.
(Presented By: Jorge Hallak, MD, PhD)

Poster #90
EPIGENETIC AND TRANSGENERATIONAL HEALTH EFFECTS OF EARLY-LIFE PATERNAL EXPOSURE TO ENVIRONMENTAL CONTAMINANTS IN A RAT MODEL
Janice Bailey, PhD[1]; Romain Lambrot, PhD[2]; Donovan Chan, PhD[2]; Serge McGraw, PhD[3]; Clotilde Maurice, PhD[4]; Arnaud Droit, PhD[5]; Astrid-Louise Deschenes, PhD[5]; Mathieu Dalvai, PhD[5]; Nancy Côté, PhD[5]; Jacquetta Trasler, MD, PhD[2] and Sarah Kimmins, PhD[2]
[1]Centre for Research in Reproduction, Development and Intergenerational Health; [2]McGill University; [3]Université de Montréal; [4]Health Canada; [5]Université Laval
(Presented By: Janice L. Bailey, PhD)

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POSTER SESSION II

Poster #91
LOSS OF TGF?-SIGNALING IN DENDRITIC CELLS LEADS TO EPIDIDYMAL UPREGULATION OF INTERFERON-INDUCED-GTPASES, EPIDIDYMITIS, ANTISPERM ANTIBODIES AND NO ORCHITIS
Fernando Pierucci-Alves, DVM[1]; Monica T. Midura-Kiela, PhD[2] and Pawel R. Kiela, DVM, PhD[2]
[1]Kansas State University; [2]University of Arizona, Tucson
(Presented By: Fernando Pierucci-Alves, DVM)

Poster #92
?-DEFENSINS PLAY A ROLE IN ANDROGEN-INDUCED WOLFFIAN DUCT MORPHOGENESIS
Camilla M. Ribeiro, PhD[1]; Lucas G. A. Ferreira, Undergraduate Student[1]; Daniel S. Thimoteo, BS[1]; Barry T. Hinton, PhD[2] and Maria Christina W. Avellar, PhD[3]
[1]Section of Experimental Endocrinology, Department of Pharmacology, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil; [2]Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, Virginia, USA; [3]Section of Experimental Endocrinology, Department of Pharmacology, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil.
(Presented By: Maria Christina W. Avellar, PhD)

Poster #93
THE EXPRESSION OF CLAUDIN-1 IN THE HUMAN SPERM IMPROVES EARLY EMBRYO DEVELOPMENT
José Manuel Lozano Sánchez, MD; Héctor Salvador Godoy Morales, MD[1]; José Francisco Gallardo Vera, PhD[2]; Antonio García Luna Vásquez, Fellow[1]; Ricardo Mera Mejía, Biology[1]; Paola Berenice Merchand Álvarez, MD[1]; Mirna Guadalupe Echavarría Sánchez, MD[1]; Miguel Tapia Rodríguez, PhD[2]; Luis Felipe Montaño Estrada, PhD[2] and Erika Patricia Rendón Huerta, PhD[2]
[1]Art Reproducción; [2]Universidad Nacional Autónoma de México
(Presented By: Antonio García Luna Vásquez)

Poster #94
SPERMATOGENESIS IN HAMSTER TESTICULAR TISSUE PLACED WITHIN AN ABDOMINAL SCROTUM MICROSURGICALLY CONSTRUCTED IN NUDE RATS
Fotios Dimitriadis, MD, PhD, FEBU[1]; Dimitrios Giannakis, Ass Professor[2]; Panagiota Tsounapi, Ast Professor[3]; Charalampos Mamoulakis, Ast Professor[4]; Georgios Semini, MD[2]; Sotiris Skouros, MD[2]; Sotiris Stavrou, MD[2]; Ioannis Giannakis, MD[2]; Atsushi Takenaka, Professor[3] and Nikolaos Sofikitis, Professor[2]
[1]Ioannina University; [2]Urology Dpt. Ioannina University, Ioannina, Greece; [3]Urology Dpt. Tottori University, Yonago, Japan; [4]Urology Dpt. Herakleion University, Crete, Greece
(Presented By: Fotios Dimitriadis, PhD)

Poster #95
ADIPONECTIN REGULATES THE DEVELOPMENT OF RAT STEM LEYDIG CELLS INTO LEYDIG CELL LINEAGE
Linxi Li, PhD; Yiyan Wang, MD; Shuyan Cao, MSc; Guimin Wang, MD; Junhui Zhang, MD; Jingjing Guo, MD; Qingquan Lian, MD and Renshan Ge, MD
The Second Affiliated Hospital, Wenzhou Medical University
(Presented By: Linxi Li, PhD)

Poster #96
COMPARISON OF TWO POPULAR VITRIFICATION DEVICES FOR HUMAN IVF
Tony Tsai, MD[1] and Huai Feng, PhD[2]
[1]Weill Medical College of Cornell University; [2]Weill Cornell Medical College
(Presented By: Tony Tsai, MD)

Poster #97
LOSS OF SPERM DNA INTEGRITY AND CHILDHOOD CANCER: IMPACT OF YOGA AND MEDITATION
Rima Dada, MD, PhD[1]; Shiv Basant Kumar, MSc[1]; Surabhi Gautam, PhD, Scholar[1] and Bhavna Chawla, Associate Professor[2]
[1]Lab For Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; [2]Retinoblastoma Clinic, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Rima Dada, MD, PhD)

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POSTER SESSION II

Poster #98
CYTOGENETIC ABNORMALITIES IN INFERTILE MALE WITH NON-OBSTRUCTIVE AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA
Moheb Mansour, MD[3]; Ezzat Elsobky, MD[2]; Yousry Moustafa, MD[3]; Moustafa Elsaied, MD[4] and Shreif Refaat, MD[5]
[1]Associate Professor of Dermatology, Andrology & STDs.; [2]Professor of Genetics Faculty of Medicine, Ain Shams University. Cairo, Egypt; [3]Professor of Dermatology, Andrology & STDs faculty of Medicine, Mansoura University, Mansoura, Egypt; [4]Associate Professor of Dermatology, Andrology & STDs faculty of Medicine, Mansoura University, Mansoura, Egypt; [5]Assistant Professor of Dermatology, Andrology & STDs faculty of Medicine, Mansoura University, Mansoura, Egypt
(Presented By: Moustafa Elsaied, MD)

Poster #99
DISCOVERY OF SEVERE OLIGOZOOSPERMIA BIOMARKERS VIA TRANSCRIPTOME ANALYSIS IN MATURE SPERM
Seyedmehdi Nourashrafeddin, PhD; Philip Sweet, BS; Archana Kishore, PhD; Andrew Georgiadis, MS; Pollock Nijole, BS; Huaiyang Jiang, MS and Alexander Yatsenko, MD, PhD
Magee Women's Research Institute, University of Pittsburgh
(Presented By: Seyedmehdi Nourashrafeddin)

Poster #100
MULTIPLE UNIQUE GENOMIC MUTATIONS FOUND IN AZOOSPERMIC AND OLIGOZOOSPERMIC INFERTILE MALES
Alexander Yatsenko, PhD; Philip Sweet; Pollock Nijole; Seyedmehdi Nourashrafeddin; Andrew Georgiadis; Archana Kishore; Huaiyang Jiang and Aleksandar Rajkovic
MWRI
(Presented By: Alexander Yatsenko, MD, PhD)

Poster #101 – WITHDRAWN

Poster #102
EXPRESSION PATTERN OF RPS6, RBM9 AND RPL10A IN RECURRENT PREGNANCY LOSS
Vidhu Dhawan, MBBS, MD[1]; Manoj Kumar, MSc, PhD[1]; Dipika Deka, MBBS, MD[2]; Vatsala Dadhwal, MBBS, MD[2] and Rima Dada, MD, PhD[1]
[1]Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; [2]Department of Obstetrics & Gynaecology, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Vidhu Dhawan, MBBS, MD)

Poster #103
THE CAENORHABDITIS ELEGANS ORTHOLOG OF HUMAN T-COMPLEX PROTEIN 11 (TCP11), M056.2, IS NECESSARY FOR MALE FERTILITY
Emily Lopes and Matthew Marcello, PhD
Pace University
(Presented By: Matthew R. Marcello, PhD)

Poster #104
POST HOC ASSESSMENT OF SOMATIC CELL CONTAMINATION IN SPERM EPIGENETIC STUDIES USING DNA METHYLATION ANALYSIS OF THE DLK1 DMR
Tim Jenkins, PhD; Kenneth Aston, PhD and Douglas Carrell, PhD
University of Utah Andrology and IVF Labs
(Presented By: Tim Jenkins, PhD)

Poster #105
EFFECT OF DIFFERENT VITRIFICATION DEVICES ON OOCYTE DNA INTEGRITY IN BOVINE OOCYTES
Huai L. Feng, PhD[1] and Tony Tsai, MD[2]
[1]Weill Medical College of Cornell University; [2]Weill Cornell Medical College
(Presented By: Huailiang Feng, PhD)

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POSTER SESSION II

Poster #106
VARIATION OF DNA FRAGMENTATION LEVELS DURING DENSITY GRADIENT SPERM SELECTION FOR ASSISTED REPRODUCTION TECHNIQUES: A NEW MALE PREDICTIVE PARAMETER OF PREGNANCY?
Monica Muratori, Nicoletta Tarozzi, Marta Cambi, Anna Lisa Iorio, Claudia Passaro, Benedetta Luppino, Marco Nadalini, Sara Marchiani, Lara Tamburrino, Luca Boni, Gianni Forti, Mario Maggi, Andrea Borini and Elisabetta Baldi
(Presented By: Elisabetta Baldi, PhD)

Poster #107
A MODIFIED MICROSCOPIC VASOEPIDIDYMOSTOMY TO TREAT EPIDIDYMAL OBSTRUCTION
Hongtao Jiang, MD[1]; Qian Yuan, MD[2]; Zengqin Liu, MD[2]; Jinan Guo, MD[2] and Zhenyu Zhou, MD[2]
[1]Shenzhen Pelple's Hospital; [2]Shenzhen People's Hospital
(Presented By: Hongtao Jiang, Sr., MD)

Poster #108
EFFECT OF YOGA ON QUALITY OF LIFE AND SEMINAL QUALITY IN MALE INFERTILITY PATIENTS WITH MAJOR DEPRESSIVE DISORDER (MDD): RANDOMIZED CONTROLLED TRIAL.
Madhuri Tolahunase, MSc[1]; Shilpa Bisht, MSc[1]; Rajesh Sagar, MD[2]; Raj Kumar Yadav, MD[3]; Saima Khan, PhD[1] and Rima Dada, MD, PhD[1]
[1]Lab for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.; [2]Department of Psychiatry, All India Institute of Medical Sciences, New Delhi, India.; [3]Department of Physiology, All India Institute of Medical Sciences, New Delhi, India.
(Presented By: Madhuri Rajaneesh Tolahunase, BAMS, MSc[Med])

Poster #109
EFFECT OF IN VITRO VITAMIN E (ALPHA-TOCOPHEROL) SUPPLEMENTATION IN HUMAN SPERM SUBMITTED TO OXIDATIVE STRESS
Luana Adami, Larissa Berladin, Bruna Lima, Mariana Antoniassi, Jessica Jeremias, Fátima Okada and Ricardo Bertolla
(Presented By: Luana Gallego Adami)

Poster #110
OPTIMIZATION OF A HIGH-THROUGHPUT SCREENING (HTS) COMPATIBLE ASSAY FOR THE SEARCH OF POTENT AND SPECIFIC SMALL-MOLECULE INHIBITORS OF THE TESTIS-SPECIFIC SERINE KINASE 1 (TSSK1) ACTIVITY
Ana Maria Salicioni, PhD and Maria Gracia Gervasi, PhD
University of Massachusetts-Amherst
(Presented By: Ana Maria Salicioni, PhD)

Poster #111
TESTICULAR TISSUE PRESERVATION IN PRE-PUBERTAL AND POST-PUBERTAL MALES
Nathalie Rives Professor, MD, PhD[1]; Aurélie Rives, Medical doctor, student[2]; Amandine Bironneau, Technician in ART[1]; Agnès Liard-Zmuda, Paediatrician Surgeon[1]; Vincent Elbaz, Paediatrician Surgeon[1]; Jean-Pierre Vannier, Paediatrician[1] and Louis Sibert, Urologist[1]
[1]Rouen University Hospital; [2]Rouen Univesity Hospital
(Presented By: Nathalie Rives)

Poster #112
OPTIMIZATION OF HORMONAL MODULATION FOR SPERMATOGENIC RECOVERY BY SPERMATOGONIAL STEM CELL TRANSPLANTATION
Gunapala Shetty, PhD[1]; Zhuang Wu, MD[2] and Marvin Meistrich, PhD[2]
[1]University of Texas M.D. Anderson Cancer Center; [2]University of Texas MD Anderson Cancer Center
(Presented By: Gunapala Shetty, PhD)

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POSTER SESSION II

Poster #113

MEN MATTER: MEN WITH INFERTILITY SHOULD HAVE A COMPREHENSIVE FERTILITY INVESTIGATION

Dana Livne Segev, MD, Urology[1]; Itay Gat, MD, Gynecology[2]; Sadea Arefin Leboni, MD[3]; Kirk Lo, MD, Urology[1]; Ethan Grober, MD, Urology[1]; Clifford Librach, MD; Gynecology[4] and Keith Jarvi, MD, Urology[1]

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(Presented By: Dana Livne-Segev, MD)

Poster #114

A MULTIDISCIPLINARY MODEL FOR EXPERIMENTAL TESTICULAR TISSUE BANKING TO GENERATE SPERMATOGENESIS IN THE FUTURE USING TRADITIONAL & ELECTRONIC MEDICAL RECORD ALERT SYSTEMS: DESCRIPTION OF A PROGRAM

Hooman Sadri-Ardekani, MD, PhD[1]; Thomas W. McLean, MD[2]; Stanley Kogan, MD[3]; Joseph Sirintrapun, MD[4]; Kathryn Crowell, Bsc[3]; Mustafa Q. Yousif, MD[5]; Steve J. Hodges, MD[6]; John Petty, MD[7]; Thomas Pranikoff, MD[7]; Leah Sieren, MD[7]; Kristen Zeller, MD[7] and Anthony Atala, MD[8]

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(Presented By: Hooman Sadri-Ardekani, MD, PhD)

Poster #115

HUMAN SPERMATOGONIAL CELLS STABILITY AFTER 24 HOURS TRANSPORTATION AND FREEZE-THAW

Hooman Sadri-Ardekani, MD, PhD[1]; Tanya Reid, Bsc[2]; Majid Mirzazadeh, MD[3]; Kathryn Crowell, Bsc[2] and Anthony Atala, MD[4]

[1]Wake Forest Institute for Regenerative Medicine & Department of Urology, Wake Forest School of Medicine, Winston-Salem, North Carolina; [2]Wake Forest Institute for Regenerative; [3]Department of Urology; [4]Wake Forest Institute for Regenerative & Department of Urology

(Presented By: Hooman Sadri-Ardekani, MD, PhD)

Poster #116

DIFFERENTIAL EFFECTS OF SILDENAFIL AND TADALAFIL IN HUMAN PENILE SMOOTH MUSCLE CELLS: NEW INSIGHTS FOR OLD MECHANISMS

Bashir Rezk; Ahmed Moustafa; Premsant Sangkum, MD; Zakaria Abd Elmageed, PhD; Suresh Sikka, PhD; Asim Abdel-Mageed, PhD and Wayne Hellstrom, MD

Tulane University

(Presented By: Bashir Mahmoud Rezk Atteia, PhD)

Poster #117

CONTEMPORARY ANALYSIS OF THE SURGICAL MANAGEMENT OF MEN WITH PEYRONIE'S DISEASE WITH HOURGLASS DEFORMITY

Faysal A. Yafi, MD, FRSCS[1]; Georgios Hatzichristodoulou, MD[2]; Julie Wang, MD[1]; James Anaissie, MD[1]; Suresh C. Sikka, PhD, HCLD[1] and Wayne J. G. Hellstrom, MD, FACS[1]

[1]Tulane University; [2]Technical University of Munich

(Presented By: Faysal A. Yafi, MD, FRCSC)

Poster #118

MIRABEGRON ELICITS SMOOTH MUSCLE RELAXATION OF HUMAN AND RAT CORPUS CAVERNOSUM TISSUES VIA NON NITRIC OXIDE-CGMP PATHWAYS

Taylor Peak[1]; Serap Gur, PhD[2]; Faysal A. Yafi, MD, FRCSC[1]; Philip J. Kadowitz, PhD[1]; Suresh C. Sikka, PhD, HCLD[1] and Wayne J. G. Hellstrom, MD, FACS[1]

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(Presented By: Taylor Peak, BA)

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POSTER SESSION II

Poster #119
LOW CALCULATED FREE TESTOSTERONE LEVELS CORRELATES SIGNIFICANTLY WITH SEXUAL DYSFUNCTION SYMPTOMS IN BORDERLINE LOW TOTAL TESTOSTERONE PATIENTS
Robert Tan, MD, MBA; Phuong Tran, MD and William Reilly, MD
Low T Center
(Presented By: Robert S. Tan)

Poster #120
NEURAL ANDROGEN RECEPTOR AND MYELIN REPAIR: ANALYZES OF ANDROGENIC POTENTIAL OF M.PRURIENS IN AGED RAT DORSAL NERVE OF PENIS REPAIR
Prakash Seppan, PhD; Karthik Ganesh Mohanraj, PhD; Muhammed Ibrahim, PhD; Ganesh Lakshmanan, MSc; Dinesh Premavathy, MSc; Sakthi Jothi Muthu, MSc and Khayinmi Shimray, Msc
University of Madras
(Presented By: Seppan Prakash, MSc, PhD)

Poster #121
SUPRAPUBIC FAT REDUCTION IN PATIENTS WITH HIDDEN PENIS
Hussein Ghanem, MD, FECSM and Ahmad Motawi, MSc, FECSM
Faculty of medicine, Cairo University
(Presented By: Ahmad Tarek Motawi, MD, MSc, FECSM)

Poster #122
HYPOGONADAL MEN WITH SEXUAL DYSFUNCTION BENEFIT FROM LPCN 1021 (ORAL TESTOSTERONE) – SOAR (STUDY OF ANDROGEN REPLACEMENT) TRIAL
Mohit Khera, MD, MBA, MPH[1]; Christina Wang, MD[2]; Jed Kaminetsky, MD[3]; Adrian Dobs, MD, MHS[4]; Irwin Goldstein ,MD[5]; Anthony Delconte, MD[6]; Nachiappan Chidambaram, PhD[7]; Satish Nachaegari, MS[8]; Mahesh Patel, PhD[8] and Martin Miner, MD[9]
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(Presented By: Mohit Khera, MD, MPH)

Poster #123
SOCIOECONOMIC FACTORS ASSOCIATED WITH PENILE PROSTHESIS RECEIPT FOR ERECTILE DYSFUNCTION IN NEW YORK STATE: ANALYSIS OF THE STATEWIDE PLANNING AND RESEARCH COOPERATIVE SYSTEM DATABASE
Ifeanyi Onyeji, BA[1]; Wilson Sui, BA[1]; Matthew Pagano, MD[1]; Aaron Weinberg, MD[1]; Maxwell James, BS[1]; Justin Matulay, MD[1]; Doron Stember, MD[2] and Peter Stahl, MD[1]
[1]Columbia University Medical Center, Department of Urology; [2]Mount Sinai Ichan School of Medicine, Department of Urology
(Presented By: Ifeanyi Cliff Onyeji, BA)

Poster #124
EFFECTS OF MICRONUTRIENT SUPPLEMENTATION ON FEMALE SEXUAL FUNCTION: A RANDOMIZED TRIAL
Fotios Dimitriadis, MD, PhD, FEBU[1]; Panagiota Tsounapi, Ast Professor[2]; Charalampos Mamoulakis, Ast Professor[3]; Dimitrios Angelis, MD[1]; Apostolos Kaponis, Ast Professor[4]; Georgios Adonakis, Ast Professor[4]; Aristomenis Gekas, MD[5]; Diamantis Dafnis, PhD[6]; Ioannis Jakumakis, MD[6]; Atsushi Takenaka, Professor[2] and Nikolaos Sofikitis, Professor[7]
[1]Urology Dpt. Ioannina University, Ioannina, Greece; [2]Urology Dpt. Tottori University, Yonago, Japan; [3]Urology Dpt. Herakleion University, Crete, Greece; [4]Obstetrics & Gynecology Dpt, Patras University, Patras, Greece; [5]Urology Dpt. "Agios Andreas" General Hospital, Patras, Greece; [6]Mediterranean Fertility Center & Genetics, Chania, Crete; [7]Ioannina University
(Presented By: Nikolaos Sofikitis, Professor)

Poster #125
COMPARISON OF SPERM CHROMOSOMAL ABNORMALITIES EVALUATED IN ONE TEENAGER AND FOUR ADULTS WITH KLINEFELTER SYNDROME
Aurélie Rives, Medical doctor, student; Véronique Duchesne, Technician in ART; Mireille Castanet, Medical doctor and Nathalie Rives, Medical doctor
Rouen University Hospital
(Presented By: Rives Aurelie)

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POSTER SESSION II

Poster #126
AWARENESS ABOUT FERTILITY OPTIONS FOR THE TRANSGENDER COMMUNITY
Dana Livne Segev, MD, Urology[1]; Raymond Fung, MD, Endocrinology[2]; Brian Hwanki Kim, Medical Student[3]; Jarvi Keith, MD, Urology[4] and Adam Millar, MD, Endocrinology[5]
[1]Department of Urology, Mount Sinai Hospital, University of Toronto, Canada; [2]Toronto East Jeneral,University of Toronto; [3]University of Toronto; [4]Department of Urology,MSH,University of Toronto; [5]Division of Endocrinology and Metabolism, MSH, University of Toronto
(Presented By: Dana Livne-Segev, MD)

Poster #127
SIMVASTATIN INDUCES CELL CYCLE ARREST AND INHIBITES PROLIFERATION OF BLADDER CANCER CELLS VIA PPAR AND ERBB SIGNALING PATHWAYS
Gang Wang, MSc[1]; Yongzhi Wang, MD[1]; Rui Cao, MSc[1]; Guofeng Qian, PhD[2]; Xinghuan Wang, MD[1] and Yu Xiao, PhD[3]
[1]Department of Urology, Zhongnan Hospital of Wuhan University, Wuhan, China; [2]Department of Endocrinology, The First Affiliated Hospital of Zhejiang University, Hangzhou, China; [3]Centre for Medical Science Research, Zhongnan Hospital of Wuhan University, Wuhan, China
(Presented By: Gang Wang, MSC)

Poster #128
NOVEL STRATEGY FOR PURIFICATION OF PROSTATE SPECIFIC ANTIGEN (PSA) FROM HUMAN SEMINAL PLASMA
Matthew Kovak, MS[1]; Rebecca Burke, MD, PhD[2]; Blake Jacks, MD[1]; Benjamin Schurhamer[1]; Bradley Houston[1]; Scott Wardlaw[1]; David Schoen[1] and Alan Diekman, PhD[1]
[1]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences; [2]Neonatal-Perinatal Medicine Clinical Postdoctoral Fellow at Baylor College of Medicine/Texas Children's Hospital
(Presented By: Matthew Kovak, MS)

Poster #129 – WITHDRAWN

Poster #130
DEPLETION OF THIOLS AND INCREASED 4-HYDROXYNONENAL DURING CRYOPRESERVATION OF STALLION SPERMATOZOA LEAD TO CASPASE ACTIVATION AND CELL DEATH
Fernando Pena, PhD[1]; Patricia Martín, DVM[1] and Cristina Ortega, PhD[2]
[1]University of Extremadura; [2]University of León
(Presented By: Fernando Juan Pena Vega, PhD)

Poster #131
EFFECTS OF PDE5 INHIBITORS ON EPIDIDYMAL RAT SPERM MOTILITY
Fotios Dimitriadis, MD, PhD, FEBU[1]; Panagiota Tsounapi, Ast Professor[2]; Charalampos Mamoulakis, Ast Professor[3]; Dimitrios Angelis, MD[4]; Apostolos Kaponis, Ast Professor[5]; Georgios Antonakis, Ass Professor[5]; Aristomenis Gekas, MD[6]; Diamantis Daphnis, PhD[7]; Ioannis Jacumakis, MD[7]; Atsushi Takenaka, Professor[2] and Nikolaos Sofikitis, Professor[4]
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(Presented By: Fotios Dimitriadis, PhD)

Poster #132
MEASUREMENT OF OXIDATION-REDUCTION POTENTIAL (ORP) AS A NEWER TOOL INDICATIVE OF OXIDATIVE STRESS IN INFERTILE MEN WITH LEUKOCYTOSPERMIA
Suresh Sikka PhD, Jaideep Toor, Lucelia Daniels, Faysal Yafi and Wayne Hellstrom
Department of Urology, Tulane University
(Presented By: Suresh C. Sikka, PhD, HCLD, CC(ABB))

Poster #133 – WITHDRAWN

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POSTER SESSION II

Poster #134
OXIDATIVE STRESS-INDUCED SPERM DNA FRAGMENTATION AND ITS ASSOCIATION WITH VARICOCELE AND SEMEN PROCESSING
Jessica Jeremias; Paula Lopes, Msc; Larissa Belardin, Bsc; Fatima Okada, PhD; Agnaldo Cedenho, PhD and Ricardo Bertolla, DVM, PhD
Department of Surgery, Division of Urology, Urology Research Center, Sao Paulo Federal University
(Presented By: Paula Intasqui Lopes, MSc, BSc)

Poster #135
PEROXIREDOXINS DIFFERENTIALLY REGULATE PHOSPHORYLATION OF PROTEIN KINASE A SUBSTRATES AND OF TYROSINE RESIDUES DURING HUMAN SPERM CAPACITATION
Donghyun Lee, BSc; Tania Morielli, MSc and Cristian O'Flaherty, PhD, DVM
McGill University and the Research Institute, McGill University Health Centre
(Presented By: Cristian O'Flaherty, PhD, DVM)

Poster #136
REGULATION OF PROTEIN PHOSPHATASE 2A DURING MOUSE SPERM CAPACITATION
Maria Gracia Gervasi, PhD and Pablo Visconti, PhD
Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts
(Presented By: Maria Gracia Gervasi, PhD)

Poster #137
RELIABILITY ESTIMATES FOR A TEST OF SPERM CAPACITATION, A FUNCTIONAL COMPLEMENT TO SEMEN ANALYSIS
G. C. Ostermeier, PhD[1]; C. Cardona, PhD[1]; T. T. Smith, PhD[2]; M. A. Moody, MS[1]; A. J. Simpson, BS[1] and A. J. Travis, VMD/PhD[3],[1]
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(Presented By: G. Charles Ostermeier, II, PhD)

Poster #138
NORMAL EJACULATES, AS DEFINED BY WHO, CAN HAVE ABNORMAL CAPACITATION ABILITY
GC Ostermeier PhD[1], C Cardona PhD[1], MA Moody MS[1], AJ Simpson BS[1], EK Seaman MD[2] and AJ Travis VMD, PhD[3],[1]
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(Presented By: G. Charles Ostermeier, II, PhD)

Poster #139
IDENTIFICATION OF BOVINE SPERM ACROSOMAL PROTEINS THAT INTERACT WITH A 32KDA ACROSOMAL MATRIX PROTEIN
Subir Nagdas, PhD; Marissa Baccas; Kimberly West; Ky'ara Carr; Christina Dejean and George Crawley
Fayetteville State University
(Presented By: Subir Nagdas, PhD)

Poster #140
SPERM FROM FERTILE MEN AND THOSE SEEKING FERTILITY EXAMS DIFFER IN THEIR ABILITY TO CAPACITATE
AJ Travis VMD, PhD[1], C Cardona PhD[2], MA Moody[2], AJ Simpson[2], EK Seaman MD[3] and GC Ostermeier PhD[2]
[1]Cornell University, Androvia LifeSciences; [2]Androvia LifeSciences; [3]Urology Group of New Jersey
(Presented By: Alexander J. Travis, VMD, PhD)

Poster #141
SONOGRAPHIC MEASUREMENT OF VISCERAL ADIPOSE TISSUE (VAT) WAS SUPERIOR TO OTHER ANTHROPOMETRIC MEASURES OF BODY FAT AS A PREDICTOR OF SPERM COUNT, MOTILITY AND DNA FRAGMENTATION (DFI).
Kevin Quach[1], Itai Gat[1], Tal Lazer[1], Sergey Moskovtsev[1], Russell Yanofsky[1], Larissa Kan-Ool Kan-Ool[1], Mozhgan Naeini[1], Howard Berger[2] and Clifford Librach[1]
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(Presented By: Kevin Quach, MSc, CCRP)

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POSTER SESSION II

Poster #142
IDENTIFICATION OF GATA4-DEPENDENT TARGET GENES IN SERTOLI CELLS BY KNOCKDOWN IN MSC-1 CELLS
Marie France Bouchard, PhD[1]; Francis Bergeron, MSc[1]; Vanessa Théberge, BSc[1]; and Robert Viger, PhD[2]
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(Presented By: Marie France Bouchard, PhD)

Poster #143
MEF2 REGULATES NRF2 GENE EXPRESSION IN MOUSE MA-10 LEYDIG CELLS
Nicholas Robert, Mickaël Di-Luoffo, PhD[1] and Jacques J. Tremblay, PhD[2]
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(Presented By: Nicholas Robert)

Poster #144
IMMUNOHISTOCHEMICAL EXPRESSION PATTERNS OF FACTORS ASSOCIATED WITH DEVELOPMENT OF PORCINE TESTIS TISSUE
Devendra Pathak, BVSc, MVSc, PhD; Awang Hazmi Awang Junaidi, DVM, MVSc; and Ali Honaramooz, DVM, PhD
University of Saskatchewan
(Presented By: Awang Hazmi Junaidi, DVM, MVSc)

Poster #145
OPTIMIZATION OF PRIMARY CULTURE CONDITIONS FOR NEONATAL PORCINE GONOCYTES
Awang Hazmi Junaidi, DVM, MVSc and Ali Honaramooz, DVM, PhD
University of Saskatchewan
(Presented By: Awang Hazmi Junaidi, DVM, MVSc)

Poster #146 – WITHDRAWN

Poster #147
ACTIVATION OF NOTCH SIGNALING IN SERTOLI CELLS IN VIVO IS DEPENDENT UPON THE PRESENCE OF GERM CELLS.
Thomas Garcia, PhD[1]; Parag Parekh, PhD[2]; Sean Kow, BS[3]; and Marie-Claude Hofmann PhD[2]
[1]University of Houston-Clear Lake; [2]UT MD Anderson Cancer Center; [3]Texas Tech University Health Sciences Center School of Medicine
(Presented By: Thomas Garcia, PhD)

Poster #148
ABLATION OF RBPJ RESCUES EARLY PRO-SPERMATOGONIA LOSS FROM ECTOPIC ACTIVATION OF NOTCH SIGNALING IN MOUSE SERTOLI CELLS
Thomas Garcia, PhD[1]; Parag Parekh, PhD[2]; Sean Kow, BS[2]; and Marie-Claude Hofmann PhD[2]
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INDEX OF ABSTRACT AUTHORS

A

Aarabi, M. 44
Abd Elmageed, Z. 116
Abdel-Mageed, A. 7, 116
Adami, L. 109
Adonakis, G. 124
Ainehchi, N. 56, 81
Altemus, A. 87
Alvau, A. 3
Amorim, . 73
Anaissie, J. 117, 55
Andrietta, J. 89
Angelis, D. 131, 124
Ann, S. 6
Antonakis, G. 131
Antoniassi, M. 11, 16, 109
Arefin Leboni, S. 113
Aston, K. 62, 104
Atala, A. 25, 114, 115
Avellar, M. 92
Awang Junaidi, A. 144, 145

B

Baccas, M. 139
Bai, Y. 31
Bailey, J. 90
Bainbridge, M. 9
Baldi, E. 106
Bale, T. 20
Barr, D. 88
Bekheirnia, M. 49
Belardin, L. 11, 64, 134
Belleannée, C. 30, 68
Benavides-Garcia, R. 75
Berger, H. 141
Bergeron, F. 68, 142
Berladin, L. 109
Berloffa Belardin, L. 8
Bertolla, R. 16, 134, 64, 109
Bhat, S. 10
Bironneau, A. 111
Bishop, C. 25
Bisht, S. 17, 108
Björkgren, I. 30
Blanchard, K. 87
Boekelheide, K. 87
Boilard, E. 30
Boisverts, A. 18
Boni, L. 106
Borini, A. 106
Bouchard, M. 142
Bourgey, M. 60
Braun, R. 1
Breton, S. 28, 29
Brimo, F. 60
Brown, T. 79
Burke, R. 128
Burnett, A. 79
Butler, P. 9

C

Calvo, É. 30
Camargo, M. 8, 16, 64
Cambi, M. 106
Campioli, E. 21
Cao, R. 127
Cao, S. 95
Cardona, C. 12, 137, 138, 140
Cardozo, K. 16
Carr, K. 139
Carrell, D. 62, 104
Carvalho, V. 16
Castanet, M. 125
Cedenho, A. 134
Cengiz, C. 9, 49
Chan, D. 44, 90
Chan, J. 20
Chan, P. 60
Chandra, P. 7
Chawla, B. 17, 97
Chen, H. 14, 24, 31, 72, 79, 82, 86
Chen, X. 32, 34, 82
Chidambaram, N. 85, 122
Cho, C. 65
Christensen, K. 44
Ciccone, I. 23
Conrad, D. 43, 73
Cooke, P. 4, 72
Costa, E. 23
Côté, N. 90
Crawley, G. 139
Crowell, K. 114, 115
Culty, M. 18, 60

D

Dada, R. 17, 41, 97, 102, 108
Dadhwal, V. 41, 102
Dafnis, D. 124
Dalvai, M. 90
Daniels, L. 47, 132
Daphnis, D. 131
De Caro, J. 88
Dejean, C. 139
Deka, D. 41, 102
Delconte, A. 85, 122
Dere, E. 87
Deschenes, A. 90
Dhawan, D. 102
Dhawan, V. 41
Diekman, A. 128
Di-Luoffo, M. 143
Dimitriadis, F. 94, 124, 131
Dobs, A. 85, 122
Dong, Y. 32
Doungkamchan, C. 1
Downey, A. 15
Droit, A. 68, 90
Duchesne, V. 125

E

Echavarría Sánchez, M. 93
Elamrawi, G. 80
Elbaz, V. 111
Ellis, L. 52
Elsaied, M. 98
Elsobky, E. 98
Emery, B. 62
Eskandari-Shahraki, M. 27
Esquivel-Franco, D. 54

F

Fantin-Domeniconi, R. 6
Fayomi, A. 5
Felice, M. 76
Feng, H. 96, 105
Feng, J. 35
Fernández-Guasti, A. 54
Ferreira, L. 92
Ferreira-Souza, A. 6

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INDEX OF ABSTRACT AUTHORS

Filice, M. 77
Forti, G. 106
Fortier, M. 30
Foster, J. 52
Fung, R. 126

G

Gabrielsen, J. 67
Gaffney, C. 53, 57
Gallardo Vera, J. 93
García Luna Vásquez, A. 93
Garcia, T. 74, 147, 148
Gat, I. 76, 77, 113, 141
Gautam, S. 17, 97
Gauthier Fisher, A. 76
Gauthier-Fisher, A. 77
Ge, R. 14, 31, 32, 33, 34, 38, 82, 86, 95
Gekas, A. 124, 131
Georgiadis, A. 99, 100
Gervasi, M. 110, 136
Ghanem, H. 121
Giannakis, D. 94
Giannakis, I. 94
Gibbs, R. 9
Godoy Morales, H. 93
Godoy, H. 46
Goldstein, I. 85, 122
Gonzalez-Peramato, P. 60
Goodson, S. 10
Greimel, P. 18
Grober, E. 113
Guan, H. 33
Guo, J. 31, 82, 86, 95, 107
Gur, S. 118
Gutiérrez-Ospina, G. 54

H

Hales, B. 15
Hallak, J. 22, 23, 48, 89
Hammond, G. 40
Hatzichristodoulou, G. 117
Hayden, T. 60
Hellstrom, W. 47, 55, 116, 117, 118, 132
Helsel, A. 2
Herati, A. 9
Hermann, B. 5, 75
Hess, R. 4
Hinton, B. 6, 92
Ho Rui Yuan, N. 73
Hodges, S. 114
Hofmann, M. 74, 147, 148
Honaramooz, A. 144, 145
Houston, B. 128
Hudson, T. 59
Hueso, A. 60
Hullin-Matsuda, F. 18
Hwang, K. 69
Hwanki Kim, B. 126

I

Ibrahim, M. 120
Intasqui Lopes, P. 8
Intasqui, P. 11, 16, 64
Iorio, A. 106

J

Jabado, N. 60
Jacks, B. 128
Jacumakis, I. 131
Jakumakis, I. 124
James, M. 58, 123
Jameson, J. 84
Jarvi, K. 76, 113
Jaworski, S. 10
Jenkins, T. 104
Jerczynski, O. 30
Jeremias, J. 109, 134
Jhangiani, S. 9
Jiang, H. 99, 100, 107
Jiménez-Sánchez, L. 54
Joly-Beauparlant, C. 68
Jones, S. 18

K

Kadowitz, P. 118
Kaminetsky, J. 85, 122
Kan-Ool, L. 141
Kao, C. 10
Kaponis, A. 124, 131
Keith, J. 126
Kenigsberg, S. 77
Kesner, J. 88
Khaki, A. 56, 81
Khan, S. 108
Khera, M. 85, 122
Khurana, N. 7
Kim, B. 28, 29
Kimmins, S. 90
Kishore, A. 99, 100
Knippschild, U. 65
Kobayashi, T. 18
Kogan, S. 114
Kohlmeier, M. 10
Kotzur, T. 75
Kovak, M. 128
Kow, S. 147, 148
Krautkramer, M. 61
Kumar, M. 41, 102
Kumar, R. 51
Kumar, S. 17, 97

L

Lacroix-Pépin, N. 30
Lakshmanan, G. 120
Lamb, D. 9, 49
Lambrot, R. 90
Lan, X. 37
Landry, M. 44
Lau, M. 21
Lazer, T. 141
Lee, D. 135
Lee, S. 19, 21
Lemaçon, A. 68
Levin, E. 4
Levy, A. 57
Li, C. 34, 38
Li, H. 65
Li, L. 31, 33, 34, 82, 86, 95
Li, S. 33
Li, W. 65
Li, X. 14, 31, 32, 33, 34, 38, 82
Lian, Q. 32, 82, 86, 95
Lian, X. 33
Liard-Zmuda, A. 111
Librach, C. 76, 77, 113, 141
Lima, A. 73

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INDEX OF ABSTRACT AUTHORS

Lima, B. 109
Lin, H. 82
Lipshultz, L. 9, 49
Liu, H. 65
Liu, J. 65
Liu, S. 82, 86
Liu, Y. 37
Liu, Z. 107
Livne Segev, D. 113, 126
Lo, K. 76, 113
Lock, T. 50
Locker, S. 4
Lopes, A. 73
Lopes, E. 103
Lopes, P. 134
Lozano Mendoza, M. 46
Lozano Sanchez, J. 46
Lozano Sánchez, J. 93
Lu, X. 6
Luppino, B. 106
Lupski, J. 9
Ly, L. 44

M

Machen, G. 59
Maggi, M. 106
Maghen, L. 76, 77
Malhotra, N. 51
Mamoulakis, C. 94, 124, 131
Manjunath, P. 27
Manku, G. 60
Mansour, M. 98
Mao, B. 31, 34
Marcello, M. 103
Marchiani, S. 106
Marcus, M. 88
Marder, M. 88
Marlowe, T. 10
Marshall, G. 5
Martin, L. 71
Martín, P. 130
Martinez-Arguelles, D. 19, 66
Matulay, J. 123
Maurice, C. 90
McClain, D. 67
McGraw, S. 90
McLean, T. 114
McMillan, L. 10
Mecklenburg, J. 75
Medrano, T. 4, 72
Meistrich, M. 112
Mera Mejía, R. 93
Merchand Álvarez, P. 93
Meyers, W. 40
Millar, A. 126
Miner, M. 85, 122
Mirzazadeh, M. 115
Mishra, S. 51
Mitchell, L. 69
Mohanraj, K. 120
Mondal, D. 7
Montaño Estrada, L. 93
Monteiro, R. 22, 23
Moody, M. 12, 137, 138, 140
Morielli, T. 135
Morris, P. 69
Moskovtsev, S. 141
Motawi, A. 121
Moustafa, A. 116
Moustafa, Y. 98
Muratori, M. 106
Musicki, B. 79
Muthu, S. 120

N

Nachaegari, S. 85, 122
Nadalini, M. 106
Naeini, M. 141
Nagdas, S. 139
Naghi, A. 18
Nagirnaja, L. 43
Nanjappa, M. 4, 72
Navarrete, F. 39
Nijole, P. 99, 100
Noordam, M. 43
Northrop, A. 52
Nourashrafeddin, S. 99, 100

O

Oatley, J. 2
O'Brien, D. 10
O'Flaherty, C. 135
Okada, F. 109, 134
Onyeji, I. 57, 58, 123
Ortega, C. 130
Orwig, K. 1, 5
Ostermeier, G. 12, 137, 138, 140
Oude Ophuis, R. 50

P

Pagano, M. 53, 57, 58, 123
Palladino, M. 42
Papadopoulos, V. 18, 19, 21, 66
Parekh, P. 74, 147, 148
Pariz, J. 22, 23, 48, 89
Park, Y. 29
Parker, M. 42
Parrish, J. 61
Passaro, C. 106
Pastuszak, A. 49
Patel, M. 85, 122
Pathak, D. 144
Peak, T. 118
Pedram, A. 4
Pena, F. 130
Pendergraft, S. 25
Pereira Cedenho, A. 8
Petty, J. 114
Phillips, J. 87
Pieri, P. 89
Pierucci-Alves, F. 91
Pilsner, J. 26
Pimenta Bertolla, R. 8, 11
Pin, G. 65
Pranikoff, T. 114
Premavathy, D. 120
Prud'homme, B. 27

Q

Qian, G. 127
Quach, K. 141

R

R. Kiela, P. 91
Rajamanickam, G. 70
Rajkovic, A. 100
Ramírez-Santos, J. 54
Ranéa, C. 23
Refaat, S. 98

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INDEX OF ABSTRACT AUTHORS

Reid, T. 115
Reilly, W. 119
Rendón Huerta, E. 93
Rezk, B. 116
Riazalhosseini, Y. 60
Ribeiro de Andrade, M. 11
Ribeiro, C. 92
Rives, A. 111, 125
Rives, N. 111, 125
Robaire, B. 15
Robert, N. 143
Roumaud, P. 71
Rozen, R. 44
Rwigemera, A. 71

S

Saad, F. 83
Sadri-Ardekani, H. 25, 114, 115
Sagar, R. 108
Salaye, L. 67
Saldiva, P. 22, 89
Salicioni, A. 110
Sangkum, P. 116
Sanguankeo, A. 13
Schoen, D. 128
Schrader, S. 88
Schrott, R. 24
Schurhamer, B. 128
Seaman, E. 12, 138, 140
Semini, G. 94
Seppan, P. 120
Sha, J. 37
Shen, Q. 33
Sheng, Y. 1
Shetty, G. 112
Shimray, K. 120
Shlush, E. 76, 77
Sibert, L. 111
Sieren, L. 114
Sikka, S. 7, 47, 55, 116, 117, 118, 132
Simon, L. 62
Simpson, A. 12, 137, 138, 140
Singh, N. 51
Sinha, K. 74
Sipilä, P. 30
Sirintrapun, J. 114
Sites, C. 26
Skouros, S. 94
Smith, J. 87
Smith, T. 137
Soeterik, T. 50
Sofikitis, N. 94, 124, 131
Song, S. 65
Spaine, D. 11
Stahl, P. 53, 57, 58, 123
Stavrou, S. 94
Steinhardt, G. 88
Stember, D. 58, 123
Stevans, A. 10
Strauss, J. 65
Su, Y. 38
Su, Z. 38
Sui, W. 58, 123
Sukhwani, M. 1
Suslik Zylbersztejn, D. 8
Sweet, P. 99, 100

T

T. Midura-Kiela, M. 91
Takenaka, A. 94, 124, 131
Talwar, S. 7
Tamburrino, L. 106
Tan, R. 78, 119
Tanrikut, C. 67
Tapia Rodríguez, M. 93
Tarozzi, N. 106
Tayyab, R. 26
Terhune-Cotter, B. 52
Terrell, M. 88
Teves, M. 65
Théberge, V. 68, 142
Thimoteo, D. 92
Thundathil, J. 70
Tibaldi, D. 11
Tolahunase, M. 108
Tong, C. 37
Toni Del Giudice, P. 8
Toor, J. 47, 132
Tran, P. 78, 119
Traore, E. 55
Trasler, J. 44, 90
Travis, A. 12, 137, 138, 140
Tremblay, J. 143
Tsai, T. 96, 105
Tsounapi, P. 94, 124, 131
Turner, T. 88

U

Uladsaheb Madarek, E. 81
Upala, S. 13

V

Vannier, J. 111
Veenboer, P. 50
Velez, M. 58
Venugopal, S. 66
Viger, R. 68, 142
Visconti, P. 3, 39, 136

W

Wainstock, T. 88
Wang, C. 85, 122
Wang, G. 31, 38, 82, 86, 95, 127
Wang, J. 117
Wang, Q. 31, 32
Wang, X. 34, 127
Wang, Y. 31, 32, 34, 82, 86, 95, 127
Wang, Z. 14
Wardlaw, S. 128
Washington, A. 6
Waxman, J. 59
Weinberg, A. 58, 123
West, K. 139
White, S. 10
Whitfield, P. 78
Wu, H. 26, 37
Wu, Q. 44
Wu, X. 33
Wu, Y. 34
Wu, Z. 112

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INDEX OF ABSTRACT AUTHORS

X

Xiao, Y. 127
Xu, B. 6

Y

Yadav, R. 108
Yafi, F. 47, 55, 117, 118, 132
Yang, H. 32
Yanofsky, R. 141
Yatsenko, A. 99, 100
Yousif, M. 114
Yu, J. 37
Yuan, K. 38
Yuan, Q. 107

Z

Zahedi, A. 56
Zeisel, S. 10
Zeller, K. 114
Zhang, J. 95
Zhang, L. 65
Zhang, Y. 79
Zhang, Z. 65
Zhou, S. 33
Zhou, Z. 107
Zhu, W. 35
Zirkin, B. 14, 24, 72, 79
Zitzmann, M. 83
Zohni, K. 76, 77
Zylbersztejn, D. 64

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ABSTRACTS

Sunday, April 3, 2016
***Poster Session I**
11:00 a.m. – 12:30 p.m.
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

1 (Oral/Poster)

SERTOLI CELL GENE THERAPY IN SERTOLI CELL ANDROGEN RECEPTOR KNOCKOUT (SCARKO) MICE

Chatchanan Doungkamchan, MD[1]; Yi Sheng, MD, PhD[2]; Meena Sukhwani, PhD[3]; Robert E. Braun, PhD[4] and Kyle E. Orwig, PhD[2]
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(Presented By: Chatchanan Doungkamchan, MD)

Introduction: Non-obstructive azoospermia with a uniform maturation arrest phenotype (NOA-MA) has poor prognosis for sperm retrieval by testicular sperm extraction (TESE). Some cases of NOA-MA can be linked to a single gene defect in somatic cells and/or Germ cells. We hypothesize that gene therapy can restore aberrant gene expression and fertility in some men with NOA-MA. Since the ethics of germline gene therapy are actively debated, our objective was to provide a proof in principle that gene therapy can correct a defective gene in Sertoli cells without germline modification. We used non-integrating adenoviral vectors to deliver a human androgen receptor (hAR) transgene to Sertoli Cell Androgen Receptor Knockout (SCARKO) mice, which have an NOA-MA phenotype.
Methods: The therapeutic vector, Adeno-hAR, contains an EF1a promoter driving EGFP and hAR expression. The Adeno-empty control vector did not have hAR. To evaluate transgene delivery, we injected Adeno-empty (2.8x106 ifu) into the seminiferous tubules of one testis. The injected and non-injected testes were evaluated 20 days later by co-staining for EGFP/VASA (germ cell marker) or EGFP/SOX9 (Sertoli cell marker). To evaluate if Adeno-hAR could restore AR expression, we injected the right testis of SCARKO mice with Adeno-hAR (2.8x106 ifu) and the left testis with Adeno-empty (2.8x106 ifu). Immunohistochemistry was done to determine EGFP and AR expression 20 days after injection.
Results: SCARKO mice were infertile; had small testes with an NOA-MA phenotype and lacked AR expression in Sertoli cells. The adenoviral vectors efficiently transduced SOX9+ Sertoli cells, but not VASA+ germ cells. Adeno-hAR restored AR expression in Sertoli cells of SCARKO mouse testes. Adeno-empty did not.
Conclusion: Sertoli cell gene therapy may have application for treating some cases of male infertility. We confirmed that non-integrating adenoviral vectors could introduce a corrective hAR transgene into Sertoli cells. Ongoing studies will quantify the risk of germline modification, map viral integration sites and evaluate fertility of untreated versus treated mice by breeding.
Funding: Magee-Womens Research Institute and Foundation and grants from the Eunice Kennedy Shriver National Institute for Child Health and Human Development (HD076412 and HD075795).

2 (Oral/Poster)

GLYCOLYSIS OPTIMIZED CONDITIONS IMPROVE THE REGENERATIVE QUALITY OF SPERMATOGONIAL STEM CELLS DURING LONG-TERM IN VITRO MAINTENANCE

Aileen Helsel and Jon Oatley, PhD
Washington State University
(Presented By: Aileen Helsel)

Introduction: Spermatogonial stem cells (SSCs) are a rare subset of the undifferentiated spermatogonial population and their activities provide the foundation for continual sperm production. A remarkable feature of SSCs is their capacity to regenerate the spermatogenic lineage following transplantation into the testes of a sterile recipient. A potential clinical application of SSC transplant is to treat infertility in cancer patients with germline elimination caused by cytotoxic effects of chemotherapy. The number of SSCs that can be collected from testicular tissue is low, thus, application is dependent on the ability to expand a primary population in vitro.
Methods: At present, culture methodologies are available for rodents and are being used as the basis for development of similar conditions for human SSCs. However, the percentage of rodent primary culture populations that possess regenerative capacity is low (1-10%) and the ability to regenerate complete colonies of spermatogenesis declines during long-term culture (4-6 months). Thus, there is need to refine culture conditions to optimize SSC maintenance and a potential avenue to address this is to match conditions to SSC bioenergetics.
Results: Based on the outcomes of RNA-Seq analysis, we discovered that expression of key genes involved in the regulation of glycolysis are up-regulated in SSCs. In the current study, we explored the impacts of altering culture conditions to favor utilization of glycolysis as the primary bioenergetics process on maintenance of SSCs in primary cultures of mouse undifferentiated spermatogonia. Conventional culture conditions include maintenance in 21% O2 and media containing free fatty acids, which promote utilization of beta-oxidation as the primary bioenergetics process. To produce glycolysis optimized (GO) conditions, we cultured cells in 10% O2 and removed free fatty acids from the media. Transplantation analyses at two-month intervals revealed that the capacity to regenerate complete colonies of spermatogenesis was improved over the six-month culture period in the GO condition compared to the conventional condition.
Conclusion: These findings indicate that matching culture conditions to the bioenergetics effects stem cell fidelity in primary cultures of mouse undifferentiated spermatogonia. Importantly, this information may be useful in devising culture conditions for SSCs of higher order mammals including humans. This research was supported by grant HD061665 awarded to JMO from the NICHD.

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3 (Oral/Poster)

THE TYROSINE KINASE FER IS RESPONSIBLE FOR THE CAPACITATION-ASSOCIATED INCREASE IN TYROSINE PHOSPHORYLATION

Antonio Alvau, MSc and Pablo Visconti, Principal Investigator
University of Massachusetts, Amherst
(Presented By: Antonio Alvau, MSc)

Introduction: Sperm capacitation comprises all the physiological changes occurring to mammalian spermatozoa during the transit through the female reproductive tract and is necessary for successful fertilization both in vivo and in vitro. At the molecular level, the process is associated with a fast activation of cAMP-dependent phosphorylation by protein kinase A (PKA). The activation of PKA leads to a slow increase in the levels of tyrosine phosphorylation, another established hallmark of sperm capacitation. Several candidates to be responsible for the increase in tyrosine have been suggested. However, due to limitations in the use of knock-out approaches for sperm research, the identity of the kinase ultimately responsible for the increased levels of phosphorylation has not been demonstrated yet.
Objective: The aim of the present research was to investigate the identity of the tyrosine kinase responsible for the capacitation-induced increase in tyrosine phosphorylation.
Methods: In order to fulfill this purpose, both pharmacological inhibitors and genetically modified murine models were used. Levels of Tyrosine phosphorylation were tested by SDS-page, with antibodies directed against phosphorylated tyrosine residues. Effect of pharmacological inhibitors on recombinant kinases in vitro was tested by the use of radioactive assay.
Results: Mice lacking PYK2 tyrosine kinase display normal capacitation-associated increase in tyrosine phosphorylation. On the other hand, sperm from mice targeted with a mutation that abolish the activity of the FER kinase (FERDR/DR), fail to undergo the increase in tyrosine phosphorylation associated with sperm capacitation. Our results indicate that FER tyrosine kinase is the tyrosine kinase responsible for the capacitation-associated increase in tyrosine phosphorylation. Interestingly, although sperm from FERDR/DR animals have significantly reduced capability to fertilize metaphase II-arrested eggs in vitro, these mice are fertile in vivo.
Conclusion: Our results suggest that FER is the kinase responsible for the regulation of the increase of tyrosine phosphorylation associated with sperm capacitation in murine.

4 (Oral/Poster)

LACK OF MEMBRANE ESTROGEN RECEPTOR ALPHA LEADS TO SPERM ABNORMALITIES, DECREASED SPERM MOTILITY AND VIABILITY, AND PROGRESSIVE INFERTILITY IN MALE MICE

Manjunatha Nanjappa, PhD[1]; Theresa Medrano, MS[1]; Seth Locker[1]; Rex Hess, PhD[2]; Ali Pedram, PhD[3]; Ellis Levin, MD[4] and Paul Cooke, PhD[1]
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(Presented By: Manjunatha Ketumaran Nanjappa, BVSc & AH, MVSc, PhD)

Introduction: 17β-Estradiol (E2) regulates male and female reproduction through its main receptor, estrogen receptor alpha (ESR1). ESR1 is expressed in rete testes, efferent ductules (EDs) and epididymis, and global loss of ESR1 (Esr1KO) causes infertility due to failure of EDs to concentrate testicular fluid, resulting in back pressure atrophy of seminiferous tubules (ST) in mice. ESR1 is mainly nuclear/cytoplasmic; however, 5% of ESR1 is palmitoylated at cysteine 451 in mice and localized to cell membranes, where it mediates rapid E2 actions.
Methods: This study investigated roles of membrane ESR1 (mESR1) in male reproduction using Esr1 knockin mice containing a mutated palmitoylation site (C451A) in ESR1 which prevented cell membrane localization; these nuclear-only estrogen receptor 1 (NOER) mice express nuclear ESR1 (nESR1) but lack mESR1. Reproductive organ weights and histology, daily sperm production (DSP), sperm morphology and motility, hormones and breeding were analyzed in 4- and 8-month-old wild-type (WT) and homozygous NOER male mice.
Results: Testes weights of NOER mice were significantly higher than controls at 4 months, but not different at 8 months. NOER testes had increased numbers of abnormal STs; this was more severe at 8 months than 4 months. Rete testes of NOER mice were dilated compared to WTs. At 8 months, DSP was reduced 62% in NOER mice compared to WTs. Epithelial height of EDs was reduced and seminal vesicle weights increased in NOER vs WT mice. Caudal epididymal sperm from NOER mice showed a 75% decrease in total and progressive motility and a 40% decrease in viability compared to WT mice at 8 months of age. Furthermore, >95% of NOER epididymal sperm exhibited morphological defects (e.g., folded or coiled tail and/or absent head) compared to WT; these defects were not seen in testis, indicating they resulted from an altered epididymal environment. Serum testosterone was increased in NOER mice versus WT mice at 4 months while LH, FSH and E2 were not different.
Conclusion: Young NOER males (6 to 12 weeks) were initially subfertile, but infertile by 5 months. Loss of mESR1 in male mice causes impaired sperm production, motility and viability, extensive sperm morphological defects and progressive infertility. These results demonstrate that membrane initiated E2/ESR1 signaling is required for normal testicular and reproductive tract function and fertility but how mESR1 and nESR1 cooperate to mediate overall E2 effects on male reproduction remains to be established.

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ABSTRACTS

5 (Oral/Poster)

LONG-TERM BRDU LABELING IDENTIFIES MITOTICALLY ACTIVE ADARK AND APALE SPERMATOGONIA IN ADULT RHESUS MACAQUE TESTES

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(Presented By: Adetunji Fayomi)

Introduction: This study challenges the dogma that Adark and Apale spermatogonia function as "reserve" and "active" stem cells during steady state spermatogenesis in adult primate testes. We hypothesize that Adark are slow cycling "active" stem cells and that this will be revealed by long-term mitotic labeling with BrdU.

Methods: BrdU was administered to adult Rhesus macaques as a single intravenous bolus or administered continuously in the drinking water for three weeks. Testes were collected for analysis three hours after the bolus of BrdU or zero, five or nine weeks after the continuous administration.

Results: In testes collected three hours after a single bolus of BrdU, 2.5% of Adark and 28% of Apale were labeled, consistent with previous reports. From those data, it is difficult to ascertain whether the low level of Adark labeling is artifact or represents the expected low level labeling of slow cycling stem cells. Therefore, we administered BrdU continuously in the drinking water for three weeks and observed that 14% of Adark and 69% of Apale spermatogonia incorporated label. These results indicate that at least a portion of Adark spermatogonia are mitotically active in adult primate testes. Similarly, at least a portion of Apale spermatogonia are relatively quiescent. After five weeks of washout, the proportion of labeled Adark increased to 24% while the proportion of labeled Apale decreased to 29%. It is not possible to determine whether the increase in labeled Adark after five weeks of washout is due to subsequent divisions of labeled Adark; reversion of labeled Apale to Adark or both. The reduction in labeled Apale between zero and five weeks of washout is presumably due to differentiation and possibly reversion to Adark. Finally, Adark and Apale labeling was reduced nearly to zero after nine weeks of washout. These results indicate that the cells that incorporated label continued to be mitotically active and/or differentiate during the 12 week course of the experiment.

Conclusion: This long-term BrdU labeling experiment allowed us to unequivocally demonstrate that Adark are mitotically active in steady state spermatogenesis. Therefore, it is necessary to revisit the generalized designation of Adark and Apale spermatogonia as "reserve" and "active" stem cells in the adult primate testis.

Funding: This work was supported by NICHD grants HD055475, HD076412 and HD075795 and the Magee-Womens Research Institute and Foundation.

6 (Oral/Poster)

PROTEIN TYROSINE KINASE 7 SIGNALS THROUGH MYOSIN II TO REGULATE PLANAR CELL POLARITY-DEPENDENT TUBULAR MORPHOGENESIS OF THE WOLFFIAN/EPIDIDYMAL DUCT, WHICH IN TURN IS IMPORTANT FOR THE ACQUISITION OF SPERM MOTILITY

Bingfang Xu, PhD[1]; Angela M. Washington, BS[1]; Raquel Fantin-Domeniconi, PhD[2]; Ana Cláudia Ferreira-Souza, MS[3]; Xiaowei Lu, PhD[1]; Sutherland Ann, PhD[1] and Barry T. Hinton, PhD[1]

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(Presented By: Bingfang Xu, PhD)

Introdcution: The Wolffian duct undergoes extensive morphogenesis to achieve an optimal length and size for sperm maturation. This process is not trivial because it results in a 1 m epididymal duct in the mouse, 3 m in the rat and 6 m in the human.

Methods: To uncover the events leading to normal morphogenesis and to examine the cellular mechanisms that regulate these events, we used a conditional knockout approach to remove protein tyrosine kinase 7 (Ptk7), a regulator of planar cell polarity (PCP) and a gene previously shown to regulate cochlea and neural plate morphogenesis, from the mesoderm (Wolffian duct epithelium and surrounding mesenchyme).

Results: Our studies showed that the Wolffian duct was characterized as: (1) having epithelial cells that divided in a random orientation relative to the elongation axis of the duct, (2) the epithelium being planar polarized, which is characterized by oriented cell elongation at basal ends and oriented cell-cell association between apical and basal ends, and polarized activity of regulatory light chain of myosin II, (3) elongating via convergent extension-type of cell rearrangements while maintaining a relatively unchanged duct diameter. Loss of Ptk7 from the mesoderm resulted in loss of all PCP characteristics and failure of cells to undergo convergent extension rearrangements resulting in a duct that had shortened length, increased lumen diameter and reduced coiling that eventually led to loss of sperm motility. However, Ptk7 did not regulate cell division nor division orientation. Furthermore, deletion of Ptk7 from mesenchymal cells surrounding the epithelium did not affect duct elongation and coiling. However, deletion of Ptk7 from epithelial cells resulted in a similar phenotype to removal of Ptk7 from the mesoderm suggesting epithelial PTK7 autonomously regulates epithelial PCP in the Woffian duct.

Conculsion: Our data supports the hypothesis that PTK7 signals through myosin II to regulate PCP to ensure that convergent extension cell rearrangements drive elongation and coiling of the Wolffian duct. Therefore, PTK7 is important for Wolffian duct morphogenesis and male fertility.

Funding: This work was supported by Eunice Shriver National Institute of Child Health and Human Development/NIH Grant RO1-HD069654 (BTH), R01-DC013773 (XL), FAPESP-2014/23382-9 (RFD) and CAPES-BEX 5874/14-9 (ACFS).

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7 (Oral/Poster)

ANDROGEN RECEPTOR DEGRADATION BY SULFORAPHANE ENHANCES THE EFFICACY OF ANTI-ANDROGENS AGAINST AGGRESSIVE PROSTATE CANCER CELLS

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(Presented By: Namrata Khurana, M.Tech)

Introduction: Androgen deprivation therapy (ADT) remains the treatment of choice for prostate cancer (PC) since PC cells utilize androgen receptor (AR) for their survival and proliferation. Although initially effective, the tumor suppressive effects of anti-androgens, e.g. Bicalutamide (BIC) and Enzalutamide (ENZ), are only transient and PC cells eventually become resistant to ADT to enable the outgrowth of Castration Resistant Prostate Cancer (CRPC). Therefore, alternative approaches to downregulate AR expression in PC cells are needed to augment the efficiency of anti-androgens. Sulforaphane (SFN), a phytochemical derived from broccoli, is known to destabilize AR. We hypothesized that simultaneous exposure to physiologically achievable concentrations of SFN would be able to sensitize PC cells to antiandrogen-induced growth suppression.
Methods: In both androgen dependent (LNCaP) and androgen independent PC cell lines (C4-2B) that express AR, we monitored the anticancer effects of BIC or ENZ in the presence or absence of SFN. Effect of drug(s) on cell growth and colonization were monitored by MTT-assays and Colony Forming Unit (CFU) assays, respectively. AR expression was measured by immunoblot analysis (IB), and its subcellular localization was measured by immunofluorescence microscopy (IFM). The expression of AR and PSA (prostate specific antigen) mRNA was determined by quantitative RT-PCR.
Results: The MTT and CFU assays revealed that co-exposure to SFN significantly ($p<0.05$) enhances the growth suppressive effect of BIC and ENZ in both LNCaP and C4-2B cells. This drug combination was also able to significantly ($p<0.005$) suppress the mitogenic effects of an androgen agonist (R1881). The IB analysis showed that SFN induces degradation of AR protein in a time- and dose-dependent manner. Within 24 hours post-exposure, SFN (10 μM) exposure could decrease AR protein levels by almost 75%. RT-PCR analysis showed that SFN enhances the efficacy of BIC in decreasing AR and PSA gene expression. The IFM studies also indicated that SFN suppresses both total AR levels and the nuclear translocation of AR following R1881 stimulation of PC cells.
Conclusion: Our findings demonstrate that co-exposure to SFN can sensitize the AR expressing PC cells to antiandrogens by rapidly degrading AR protein levels. Therefore, this safe phytochemical may be used as an adjuvant therapy with ADT to suppress the recurrence of CRPC.

8 (Oral/Poster)

CYSTEINE-RICH SECRETORY PROTEIN 3 (CRISP-3) IS A CANDIDATE BIOMARKER OF TESTICULAR DYSFUNCTION IN ADOLESCENTS WITH VARICOCELE

Larissa Berloffa Belardin, BSc; Mariana Camargo, MSc; Paula Intasqui Lopes, MSc; Paula Toni Del Giudice, PhD; Daniel Suslik Zylbersztejn, MD, PhD; Ricardo Pimenta Bertolla, DVM, PhD and Agnaldo Pereira Cedenho, MD, PhD
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(Presented By: Larissa Berloffa Belardin, BSc)

Introduction: Varicocele, the presence of varicose veins in the pampiniform plexus with retrograde blood flow, initiates at puberty onset. Although many studies have associated varicocele to a progressive decline in male fertility, mainly due to testicular hyperthermia and decreased removal of toxic metabolites, underlying molecular mechanisms are not understood. In fact, adolescents with varicocele may or may not present infertility in adulthood. We have previously demonstrated alterations in the seminal plasma proteome in the presence of varicocele-induced testicular dysfunction in adolescents. We wished to validate these proteins to verify their potential in separating an infertility-defining varicocele from a "silent" varicocele. Thus, our objective was to verify seminal levels of Deoxyribonuclease 1 (DNase I), Ig gamma-3 chain C region (IGHG3), Lipid phosphate phosphohydrolase 1 (PAP2-alpha), 45 kDa calcium binding protein (Cab45), and Cysteine-rich secretory protein 3 (CRISP-3) in adolescents with and without varicocele.
Methods: 61 adolescents, between 15-19 years old, with full sexual maturity, were divided into 3 groups: controls without varicocele (C); with varicocele and normal semen (VNS); with varicocele and altered semen (VAS). Samples were centrifuged and the supernatant seminal plasma collected. Thenceforth, 50 μg of protein was utilized for Western blotting for quantification of DNase I, IGHG3, PAP2-alpha, Cab45, and CRISP-3 (normalized to Epididymis Secretory Sperm Binding Protein Li 67p [DJ-1]). Groups were compared using ANOVA (post hoc Sidak) or Kruskal-Wallis (post hoc Games Howell) ($p<0.05$).
Results: Results are presented in Table 1. VAS patients presented lower sperm concentration and morphology. DNase I was underexpressed in the VNS group, Cab45 was underexpressed in both varicocele groups, and CRISP-3 was overexpressed in the VAS group.
Conclusion: The seminal plasma proteome reflects alterations in testicular function in the adolescent varicocele. CRISP-3 is a candidate biomarker of testicular dysfunction in adolescents with varicocele, and in the future may assist in determining medical conduct for early surgical intervention.
Funding: São Paulo Research Foundation (2014/11636-6).

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Table 1. Semen analysis and seminal plasma levels of DNase1, IGHG3, PAP2-Alpha, Cab45 and CRISP3. Groups were compared by ANOVA followed by a Sidak *post-hoc test* (values presented as mean; standard deviation), unless otherwise noted.

	C (n=20)	VNS (n=22)	VAS (n=19)	*p*
Age (years)	16.5; 2.00	16.0; 2.00	16.0; 1.00	[Q] 0.595
Abstinence (days)	3.5; 1.40	4.0; 2.00	3.0; 2.00	[Q] 0.289
Sperm concentration (x10^6/mL)	80.5; 50.49 [a]	96.1; 60.94 [a]	13.4; 11.25 [b]	<0.00001 *
Progressive motility (%)	64.5; 10.50	58.0; 10.00	56.0; 16.00	[Q] 0.122
Total motility (%)	65.5; 7.25	60.1; 12.18	60; 12.65	0.201
Morphology (% normal)	9.5; 3.80 [a]	9.0; 4.00 [a]	2.0; 4.00 [b]	[Q] <0.00001 *
DNase I (a.u.)	1.8; 2.29 [a]	0.2; 0.33 [b]	1.9; 2.64 [a]	0.013 *
IGHG3 (a.u.)	1.1; 0.81	2.5; 6.36	1.4; 1.39	0.814
PAP2-Alpha (a.u.)	0.9; 1.99	0.8; 0.93	0.8; 1.04	0.789
Cab45 (a.u.)	1.4; 2.34 [a]	0.2; 0.25 [b]	0.1; 0.16 [b]	0.007 *
CRISP-3 (29KDa) (a.u.)	1.7; 1.96 [a]	1.3; 1.73 [a]	84.1; 94 [b]	<0.00001 *
CRISP-3 (31KDa) (a.u.)	2.3; 2.06 [a]	2.6; 5.36 [a]	187.8; 344.96 [b]	<0.00001 *

[Q] – Nonparametric Kruskal-Wallis test, Games Howell *post hoc* test. Values expressed in median; interquartile range (IR).
* – statistically significant difference.
Different superscript letters in a same row indicate a significant difference in the *post-hoc* test.
(a.u.) – arbitrary units, normalized to the testicular housekeeping protein DJ-1.

9 (Oral/Poster)
IFT140 IS A NOVEL CANDIDATE GENE FOR IMPAIRED SPERMATOGENESIS: IDENTIFICATION BY WHOLE EXOME SEQUENCING AND VALIDATION WITH SANGER SEQUENCING

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Baylor College of Medicine
(Presented By: Amin S. Herati, MD)

Introduction: One-third of male factor infertility cases are idiopathic and are likely caused by genetic factors. Non-obstructive azoospermia (NOA) is characterized by the absence of sperm in the ejaculate secondary to the lack of sperm production in the testicle. The aim of our study was to identify novel variants implicated in the pathogenesis of NOA through whole-exome sequencing (WES).
Methods: Homozygosity mapping with WES was performed using the NimbleGen SeqCap EZ Exome V3 sample preparation kit and the Illumina HiSeq 2000 next-generation sequencing platform to sequence the exomes of two siblings with NOA from a consanguineous family. Genetic mutations that passed filter criteria were validated using Sanger sequencing. We identified one candidate gene, Intraflagellar Transport Protein 140 (IFT140), based on its expression pattern in testes according to the Human Protein Atlas, as well the mutation's damage prediction based on two online bioinformatics resources. We subsequently performed Sanger sequencing on 114 NOA and 43 fertile men.
Results: WES identified 442 variants in the index patients. Four (0.9%) of these variants passed filter criteria, one of which was a six-nucleotide deletion (HG38, chr16:1,519,961 TCTTGGC>T) in exon 22 of IFT140. Family segregation showed that this mutation was homozygous in the two brothers who underwent WES, as well as in a third brother with NOA and their sister. This mutation was not identified in the 1000 Genome Project and was predicted to be deleterious by the bioinformatics tools, MutationTaster and PROVEAN. The Exome Aggregation Consortium database of 60,706 genomes was also queried which identified a minor allele frequency of 0.1%. Sanger sequencing validated the mutation in this family and identified it as a heterozygous mutation in 1 (0.9%) of the 114 NOA men and none of the 43 fertile controls in our cohort. Testis biopsy interpretation of this unrelated patient showed hypospermatogenesis with a clinical history significant for three failed cycles of in vitro fertilization (IVF).
Conclusion: We identified a small indel mutation in IFT140 as a novel candidate variant for infertility. This mutation was detected in our cohort of NOA men in addition to the index family, with a prevalence of 0.9%, which is higher than the minor allele frequency in an unselected general population. Further studies are required to determine the role of IFT140 in spermatogenesis.

10 (Oral/Poster)
CASANOVA: A MULTICLASS SUPPORT VECTOR MACHINE MODEL FOR THE CLASSIFICATION OF HUMAN SPERM MOTILITY PATTERNS

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(Presented By: Summer Goodson, PhD)

Introduction: Hyperactivated motility in sperm is required for normal fertilization. Traditionally, the percentage of hyperactivated human sperm assessed by computer-assisted sperm analysis (CASA) has been reported based on threshold gates for multiple kinematic parameters that reflect hyperactivated motility patterns. We previously reported a novel CASA-based approach, CASAnova, for identifying multiple patterns of sperm motility in mouse. This approach uses multiple support vector machine (SVM) equations based on standard CASA parameters to characterize the distribution of motility patterns in large populations of sperm. We have now adapted CASAnova for the analysis of human sperm motility.
Methods: Using 2,817 visually classified sperm tracks obtained from 14 individuals, we generated a web-based program that incorporates kinematic parameters of individual sperm into five SVM equations. These equations are used to classify sperm tracks into one of five categories of motility (progressive, intermediate, hyperactivated, slow, and weakly motile) with an overall accuracy exceeding 89%.
Results: Comparison of visually-assessed levels of hyperactivation versus levels generated by CASAnova or kinematic thresholds shows that CASAnova estimates of hyperactivated motility more closely reflect visual estimates than those calculated by gating. When applied to populations of sperm incubated in capacitating vs. non-capacitating conditions, CASAnova accurately detects the absence of hyperactivated motility in non-capacitated sperm, as well as a reduction in intermediate and progressive motility. These results indicate that CASAnova accurately detects changes in sperm motility when signaling pathways controlling normal sperm capacitation are perturbed. Furthermore, motility profiles of sperm from men harboring two minor alleles of the single nucleotide polymorphism rs12676 (located in the gene encoding choline dehydrogenase), reveal a delay in the onset of hyperactivation, a phenotype than cannot be seen using traditional gating. CASAnova allows for robust, accurate and detailed analyses of sperm motility patterns. The sensitivity of this method facilitates the detection of defects that are not seen with traditional approaches for assessing sperm motility, indicating its potential utility in future clinical

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applications that evaluate male infertility.

11 (Oral/Poster)
THE EFFECT OF ORCHIECTOMY ON FUNCTIONAL ASPECTS OF SPERM AND OXIDATIVE STRESS OF SEMINAL PLASMA FROM PATIENTS WITH TESTICULAR TUMORS

Maria Beatriz Ribeiro de Andrade, Paula Intasqui, Larissa Belardin, Mariana Antoniassi, Danielle Tibaldi, Ricardo Pimenta Bertolla and Deborah Spaine
Sao Paulo Federal University
(Presented By: Maria Beatriz Ribeiro De Andrade, BSc)

Introduction: Testicular tumors, although rare, are the most frequent tumors in young men of reproductive age. Treatment is usually achieved by orchiectomy, followed or not by adjuvant therapy, with survival rates of 95% after five years. Because adjuvant therapy is often gonadotoxic, there is a concern with reproductive life. Currently, sperm banking is the only option that guarantees future fertility in these men. However, it remains to be answered if the best sample to cryopreserve is the pre- or the post-orchiectomy sample. Therefore, we wished to verify semen quality, sperm functional traits, and semen oxidative stress, before and after orchiectomy, and suggest the better time to perform sperm banking in patients with testicular tumors.
Methods: This prospective study was carried out including 24 patients with testicular germ cell tumors, who provided one semen sample before orchiectomy of the affected testis, and one at least 15 days after the surgery. Following semen analysis, an aliquot was used for analysis of (i) sperm DNA fragmentation (alkaline Comet assay, sperm were classified as high DNA integrity [Class I] to high DNA fragmentation [Class IV]); (ii) acrosome integrity (PNA-FITC); and (iii) mitochondrial activity (colorimetric staining of the midpiece, classified as Grade I [all mitochondria active] to IV [all mitochondria inactive]. Seminal plasma MDA levels were measured as markers of oxidative stress. Pre- and Post-orchiectomy samples were compared using a paired Student's T test (normal distributions) or a paired Wilcoxon test, when appropriate ($p<0.05$).
Results: Results are presented in table 1. A significant decrease in DNA fragmentation ($p=0.019$) and oxidative stress ($p=0.014$), and an increase in mitochondrial activity ($p= 0.048$) was observed after orchiectomy.
Conclusion: Based on our findings, the better time for sperm banking in patients with testicular tumors is after orchiectomy. Although cryopreservation of pre-orchiectomy samples remains an important conduct to preserve reproductive potential, post-orchiectomy samples should be preferred for assisted reproductive treatments.

Table 1. Value of seminal analysis and DNA fragmentation (Comet), mitochondrial activity (DAB), acrosome integrity (PNA – FITC) and lipid peroxidation (TBARS). Value presented as mean; standard deviation.

	Pre - orchiectomy	Post - orchiectomy	p
Volume (mL)	3.0; 1.24	3.2; 2.46	0.620*
Progressive (%)	51.9; 12.03	50.7; 10.16	0.819
Non-progressive (%)	42.6; 12.76	44.0; 9.79	0.646
Concentration (x 10^6/mL)	26.7; 24.93	24.2; 27.12	0.227*
Morphology (%)	4.4; 2.50	4.4; 2.10	0.714
Comet I (%)	7.0; 11.00	6.5; 7.00	0.36
Comet II (%)	62.5; 12.00	72.0; 18.00	0.019*
Comet III (%)	22.5; 8.00	14.5; 9.00	0.028*
Comet IV (%)	5.0; 8.00	3.0; 5.00	0.172
DAB I (%)	1.0; 3.00	1.0; 3.00	0.612
DAB II (%)	65.5; 29.00	65.0; 17.00	0.075
DAB III (%)	34.2; 15.32	28.1; 7.71	0.048*
DAB IV (%)	4.5; 7.00	4.0; 4.00	0.710
Intact acrosomes (%)	80.9; 8.66	80.4; 8.39	0.521
MDA (ng/ml)	9.5; 4.38	7.8; 2.79	0.014*

* - Significant difference
* - Nonparametric Wilcoxon test. Value present as median; interquartile range

12 (Oral/Poster)
CONSISTENT DIFFERENCES AMONG MEN IN CAPACITATION TIMING COULD PERSONALIZE IUI/ART

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(Presented By: Alexander J. Travis, VMD, PhD)

Introduction: To become fertilization competent, sperm must first capacitate. This process depends on changes in membrane lipids, including removal of sterols and redistribution of the ganglioside GM1. Here, we examine the timing of capacitation among and within different individuals. Reliable differences in capacitation timing could be used to personalize IUI/ART protocols.
Methods: Semen samples from consenting men were liquefied, washed and aliquots incubated under non-capacitating (NC) or capacitating (CAP) conditions. Samples from fertile men (pregnant partner or recent father) and samples from men seeking fertility exams were incubated for three hours, fixed and analyzed immediately (day0) or after overnight incubation in fix (day1). In another trial, NC and CAP samples were incubated for three hours or 24 hours prior to fixation. Capacitation was assessed using localization of GM1 (Cap-ScoreTM).
Results: 102 samples from 36 fertile men were evaluated at day0 and day1. An increase in Cap-Score was observed in 81% (83/102) of CAP samples, with 44% (45/102) increasing more than 1 SD (7%). In 17 men seeking fertility treatment, 29% (5/17) increased 7% or more. To determine whether this change was physiological or an artifact of being in fix overnight, semen samples from nine fertile men were analyzed at day0, day1 and after 24 hours of incubation in medium and then fixed. All NC samples were equivalent (19±2, 23±2 and 20±1%) and were different from the CAP samples (28±1, 34±2 and 31±2%). Day1 CAP was significantly greater than Day0 CAP ($p=0.03$), but Cap-Scores for samples incubated overnight in fix or in capacitating media were the same ($p=0.33$). Consistent with prior literature, these data show that membrane changes involved in capacitation still occur in certain fixatives. These data suggest that sperm achieve capacitation at different times in different ejaculates. To see if this was reproducible for an individual, 91 samples from 25 fertile men were classified as either early or late capacitators (day1-day0>7). The average concordance of change within donors was 76%, showing that capacitation timing was highly consistent within men.
Conclusion: These data show that capacitation timing differed consistently among men. Determining capacitation rates could eventually lead to personalized management of infertility, including optimal times for IUI relative to ovulation and capacitation times prior to co-incubation of sperm and oocytes for IVF.
Funding: Funded by Androvia LifeSciences.

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ABSTRACTS

13

ASSOCIATION BETWEEN TESTOSTERONE, SEX HORMONE-BINDING GLOBULIN, AND NONALCOHOLIC FATTY LIVER DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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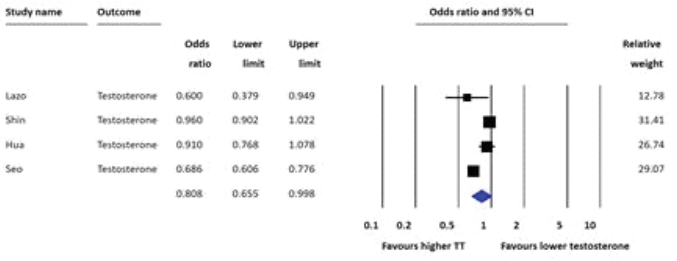
(Presented By: Sikarin Upala, MD)

Introduction: Insulin resistance is a major risk factor for development of nonalcoholic fatty liver disease (NAFLD). Several studies have shown that low sex hormone-binding globulin (SHBG) and testosterone levels are significantly associated with insulin resistance, metabolic syndrome, and type 2 diabetes. However, association between SHBG, serum testosterone and NAFLD is still controversy. We performed a systematic review and meta-analysis to assess this association.
Methods: A comprehensive search of the databases of MEDLINE and EMBASE was performed from inception through December 2015. The inclusion criterion was the original observational study that compared prevalence of NAFLD and level of testosterone and SHBG. We included only studies that adjusted for confounders. Outcome was the pooled adjusted odds ratio (OR) of NAFLD between high and low serum testosterone or SHBG. Two authors performed literature search and quality assessment. Pooled OR and 95% confidence interval (CI) were calculated using a random-effect. The between-study heterogeneity of effect-size was quantified using the Q statistic and I2.
Results: The initial search yielded 148 articles. Nine articles underwent full-length review and data was extracted from four observational studies. All studies had cross-sectional designs, involving totally 1,772 participants. NAFLD was ascertained by ultrasound without other identifiable causes of chronic liver disease. There was significantly lower prevalence of NAFLD in higher serum testosterone with pooled OR of NAFLD of 0.81 (95%CI 0.66-0.99). The statistical between-study heterogeneity (I2) was 88%, P=0.001. In the meta-analysis comparing SHBG level, there was no significant difference in prevalence of NAFLD between higher and lower SHBG with pooled OR of NALFD of 0.39 (95%CI 0.15-1.04).
Conclusion: Our meta-analysis demonstrated that higher serum testosterone level is significantly associated with lower prevalence of NAFLD after adjusting for confounding factors. This finding suggests a role of testosterone in insulin resistance and ectopic fat accumulation. Future prospective study addressing the causal relationship between serum testosterone level and NAFLD should be pursued.



14

REGULATION OF SEMINIFEROUS TUBULE-ASSOCIATED STEM LEYDIG CELLS IN ADULT RAT TESTES

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(Presented By: Xiao-Heng Li, MS)

Introduction: Testicular Leydig cells are the primary source of testosterone in males. Adult Leydig cells have been shown to arise from stem cells present in the neonatal testis. Once established, adult Leydig cells turn over only slowly during adult life; but when these cells are eliminated experimentally from the adult testis, new Leydig cells rapidly reappear. As in the neonatal testis, stem cells in the adult testis are presumed to be the source of the new Leydig cells. As yet, the mechanisms involved in regulating the proliferation and differentiation of these stem cells remain unknown.
Methods: We developed a unique in vitro system of cultured seminiferous tubules to assess the ability of factors from the seminiferous tubules to regulate the proliferation of the tubule-associated stem cells, and their subsequent entry into the Leydig cell lineage. The proliferation of the stem Leydig cells was stimulated by factors that include DHH, FGF2, PDGFBB, activin and PDGFAA. Suppression of proliferation occurred with TGFβ, androgen and PKA signaling. The differentiation of the stem cells was regulated positively by DHH (Desert hedgehog), lithium-induced signaling and activin; and negatively by TGFβ, PDGFBB, FGF2, Notch and Wnt signaling.
Results: DHH was found to function as a commitment factor, inducing the transition of stem cells to the progenitor stage and thus into the Leydig cell lineage. Additionally, CD90 was found to be a unique stem cell surface marker that can be used to obtain purified stem cells by flow cytometry.

15

ZINC TRANSPORTERS IN MALE GERM CELLS ARE AFFECTED BY TREATMENT OF MALE RATS WITH CYCLOPHOSPHAMIDE (CPA)

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(Presented By: Anne Marie Downey, BSc)

Introduction: Zinc is required for numerous enzymes and DNA binding proteins involved in oxidative stress defence and DNA damage repair. Adequate zinc levels are required for male germ cell development as zinc deficiency leads to increased oxidative stress, DNA damage and apoptosis. Zinc transport is accomplished by two families of zinc transporters: the ZIPs and the ZnTs that increase and decrease cytosolic zinc levels, respectively. However, very little is known about zinc transport in the testis. Furthermore, whether cytotoxic agents such as CPA, an alkylating agent and known male germ cell toxicant, can affect zinc transport

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and homeostasis is unknown. We hypothesize that zinc transporters are important in male germ cells and are involved in germ cell response to CPA treatment.
Methods: Adult male SD rats were gavaged daily with saline or 6 mg/kg CPA for four weeks; pachytene spermatocytes (PS) and round spermatids (RS) were collected by unit gravity sedimentation. Whole genome gene expression microarrays were used to determine the basal and post-CPA treatment expression of zinc transporters. Validation was done by qRT-PCR and proteins were assessed by western blots. Zinc uptake in germ cells was monitored using live cell imaging with a zinc fluorescent probe (Fluozin-3AM).
Results: The level of expression of members of both families of transporters varied greatly between PS and RS. RS had a greater increase in fluorescent zinc probe signal over time than PS, suggesting that they take up more zinc. CPA treatment significantly increased the expression of 4 members of the ZIP family (ZIP5, ZIP6, ZIP13 and ZIP14) in PS but not in RS. qRT-PCR confirmed that transcripts for ZIP5 and ZIP14 were significantly increased in PS after CPA treatment. ZIP5 protein concentration was also increased in PS but not in RS. PS from CPA treated animals took up more zinc over time than controls. Consistent with the expression data, there was no significant increase in zinc uptake in RS from CPA treated animals compared to controls.
Conclusion: In summary, PS and RS showed differential expression of zinc transporters and zinc uptake, likely reflecting stage specific zinc requirements. In PS CPA treatment increased the expression of members of the ZIP family, responsible for increasing cytosolic zinc levels, and increased zinc up-take. These results indicate that CPA alters zinc homeostasis; we propose that this is due to increased oxidative stress and DNA damage.
Funding: Supported by CIHR.

16

SMOKING ALTERS SPERM FUNCTION AND THE PROTEOME PROFILE IN MEN WITH VARICOCELE

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(Presented By: Mariana Pereira Antoniassi, BSc, MSc)

Introduction: Varicocele is the main treatable cause of male infertility, and affects 15%–25% of adult men, 35% of men with primary infertility and 80% of men with secondary infertility. It is characterized by testicular venous stasis, heat and oxidative stress, hypoxia, and decreased blood renewal, with accumulation of toxic substances, which renders varicocele a likely potentiator of environmental effects to the testes. The cigarette has a complex mixture of components, such as cadmium and nicotine, that accumulate in the testis. In this study we wished to evaluate the effects of smoking on sperm functional quality and proteomic profile in men with varicocele.
Methods: Sperm functional tests were performed on patients with varicocele (24 non-smoking and 25 smoking patients): evaluation of DNA fragmentation (alkaline Comet assay); mitochondrial activity (DAB staining) and acrosomal integrity (PNA binding). The remaining semen volume was centrifuged and seminal plasma utilized for proteomic analysis (LC-MS/MS). Univariate statistics were performed using a Student's t-test for unpaired samples or a Mann-Whitney test. Multivariate analysis was performed through a discriminant model. Proteins significant in both analyses were considered of interest. Differentially expressed proteins were used for functional enrichment analysis of Gene Ontology, KEGG, and Reactome categories. An alpha of 5% was adopted for the analyses.
Results: Semen results are presented in table 1. 421 proteins were identified and quantified, of which one was exclusive, 70 were underexpressed and 21 were overexpressed in smokers. Functional analysis revealed the enrichment of positive regulation of arachidonic acid secretion and binding, negative regulation of mature B cell apoptosis process and positive regulation of prostaglandin secretion involved in immune response in the smoker group.
Conclusion: Cigarette smoking is associated with an inflammatory state in the semen, as demonstrated by enriched proteomic pathways. This effects an alteration in sperm functional quality, which is characterized by decreased acrosome integrity and mitochondrial activity as well as by increased nuclear DNA fragmentation.
Funding: CNPq (160857/2012-0) (Brazil).

Table 1. Semen analysis and sperm function of smokers and nonsmokers with varicocele. Groups were compared by Student's t test, except "Comet class I, IV and DAB class I", compared by Mann-Whitney test (p<0.05). Values are presented as mean; standard deviation.

	Varicocele non-smoker (n=24)	Varicocele smoker (n=25)	p
Age (years)	32.8; 5.60	35.5; 4.21	0.067
Volume (mL)	3.8; 1.31	3.0; 1.19	0.027 *
Progressive (%)	53.6; 8.87	41.3; 16.20	0.002 *
Non-Progressive (%)	5.4; 2.32	6.6; 3.83	0.202
Sperm Concentration ($x10^6$/mL)	82.3; 46.24	63.9; 55.62	0.217
Morphology (%)	7.9; 2.83	4.8; 3.65	0.0017*
Comet class I (%)	19.3; 15.68	1.7; 4.28	<0.0001 *
Comet class II (%)	69.9; 16.10	57.0; 16.64	0.0085*
Comet class III (%)	9.8; 9.66	32.8; 14.14	<0.0001 *
Comet class IV (%)	1.0; 2.51	8.4; 6.94	<0.0001 *
Intactac acrosome (%)	72.8; 10.73	63.8; 15.6	0.024 *
DAB class I (%)	16.5; 14.90	4.0; 5.37	0.0003 *
DAB class II (%)	58.2; 12.63	48.6; 16.95	0.030 *
DAB class III (%)	20.5; 6.23	36.0; 14.29	<0.0001 *
DAB class IV (%)	4.6; 2.71	11.3; 7.32	0.0001*

* - Statistically significant difference

17

PATERNAL LIFESTYLE FACTORS ASSOCIATED WITH SPERM DNA DAMAGE

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(Presented By: Surabhi Gautam, PhD Scholar)

Introduction: Factors such as ageing, environmental toxicants like nicotine, pesticides or alcohol consumption may initiate DNA strand breakage in the spermatozoa of affected males, eventually leading to a mutation in the embryo. Sperm DNA damage is associated with poor assisted reproductive technique (ART) outcome, birth of offspring with congenital malformations, recurrent spontaneous abortions after assisted and spontaneous

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conception and even childhood cancers. Thus, this study was planned with an aim to analyse sperm DNA quality in the fathers of children with Non-Familial Sporadic Heritable Retinoblastoma (NFSHRb).

Methods: 75 fathers of children with Retinoblastoma and 75 controls were enrolled and DNA Fragmentation Index (DFI) by Sperm Chromatin Structural Assay, Reactive Oxygen Species (ROS) by Chemiluminicence Assay and 8-hydroxy-2'-deoxyguanosine (8-OHdG) by ELISA were calculated. Ethical clearance was obtained for the study.

Results: The mean ages of cases and controls were 33.17±11.2yrs and 28.5±4.54yrs respectively. The semen analysis of both cases and controls showed no remarkable differences. Seminal mean ROS levels, DFI levels and 8-OHdG levels were significantly higher in cases as compared to controls. The offsprings of fathers who smoke, pesticide exposed and alcohol consumers are at more risk of developing Rb, Odds Ratio (OR) 6.5, 95% CI: (1.4-28.8);(p=0.028*).

Conclusion: The results show that sperm DNA damage may be a cause of retinoblastoma, and paternal factors may play a critical role in embryonic development. Majority of mutations arise during cell replication and as sperm has a limited capacity for oxidative DNA damage detection and repair, thus is highly susceptible to accumulation of mutations. In a study from our lab, we have documented that lifestyle interventions (yoga/meditation) may improve DNA integrity and thus may reduce incidence of childhood cancer.

18

STIMULATORY EFFECTS OF COMBINED ENDOCRINE DISRUPTORS ON MA-10 LEYDIG CELL STEROID PRODUCTION AND LIPID HOMEOSTASIS

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(Presented By: Steven Jones, BSc, MSc)

Introduction: Previous work in our laboratory demonstrated the ability of in utero exposure to a mixture of the phytoestrogen, Genistein (GEN), and plasticizer, DEHP, to induce long term alterations in gene and protein expression that are substantially different from individual exposures. Recent data identified fetal-type and adult Leydig cells, as well as their progenitors, as sensitive targets for low dose endocrine disruptor (ED) mixtures.

Methods: To further investigate the direct effects of GEN and DEHP and elucidate specific mechanisms of toxicity, MA-10 Leydig cells were exposed in-vitro to varying concentrations of GEN and MEHP, the principle bioactive metabolite of DEHP. 10 μM combined GEN + MEHP had a stimulatory effect on basal MA-10 progesterone production. Consistent with increased androgenicity, the mRNA of steroidogenic and cholesterol mediators Star, Cyp11a, Srb1 and Hsl, as well as upstream orphan nuclear receptors Nr2f2 and Sf1 were all significantly increased uniquely in the mixture treatment group.

Results: Insl3, a sensitive marker of Leydig endocrine disruption and cell function, was significantly decreased by combined GEN + MEHP. Lipid analysis by high-performance thin layer chromatography demonstrated the ability of combined 10 μM combined GEN + MEHP, but not individual exposures, to increase levels of several neutral lipids and phospholipid classes, indicating a generalized deregulation of lipid homeostasis. Further investigation by qPCR analysis revealed a concomitant increase in cholesterol (Hmgcoa) and phospholipid (Srebp1c, Fasn) mediator mRNAs, suggesting the possible involvement of upstream LXRα agonism.

Conclusion: These results suggest a deregulation of MA-10 Leydig function in response to a combination of GEN + MEHP, suggesting synergism and in some cases bi-phasic effects. We propose a working model for low doses GEN + MEHP exposure that likely involves LXR agonism and direct or indirect activation of other transcription factors. Taken more broadly, this research highlights the importance of assessing the impact of ED mixtures in multiple toxicological models across a range of environmentally relevant doses.

19

IN UTERO EXPOSURE TO DEHP ALTERS STEROIDOGENESIS IN THE ADULT MALE OFFSPRING: REVERSIBILITY OF THE EFFECTS USING PHARMACOLOGICAL MEANS

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(Presented By: Sunghoon Lee, BSc, MSc)

Introduction: Phthalates are plasticizers used in the production of polyvinyl chloride products. Di-(2-ethylhexyl) phthalate (DEHP) is one of the most manufactured plasticizers and is found in food products, cosmetics, furniture, and medical equipment. DEHP is a non-covalently bound additive contributing to the flexibility and durability of plastic products, from which it can leach out into the environment.

Methoeds: To study the long-term effects of in utero exposure to DEHP, we exposed pregnant Sprague-Dawley rats by gavage from gestational day (GD) 14 until birth. This fetal exposure resulted in decreased levels of testosterone and aldosterone in the adult male offspring. In the adrenal gland, studies revealed that aldosterone levels remained decreased in the elderly rat. Interestingly, administration of a low-sodium diet, a stimulus for aldosterone production, resulted in increased aldosterone levels that were similar to controls, suggesting that the zona glomerulosa, the main site of aldosterone biosynthesis, had retained its steroidogenic response to circulating levels of sodium. The goal of the current study was to ascertain whether DEHP alters the capacity of steroidogenic cells to produce testosterone and corticosterone in response to hormone stimulation. We treated pregnant dams with oil, 1 (low), or 300 (high) mg DEHP/kg/day from GD14 until birth. At postnatal day 60, male offspring were injected with human chorionic gonadotropin (hCG; 100 I.U.) or adrenocorticotropic hormone (ACTH; 500 μg/kg) one hour prior to blood collection.

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Results: Serum analysis revealed that DEHP exposure altered circulating levels of testosterone and corticosterone produced in the presence of physiological circulating levels of LH and ACTH. Treatment of the male offspring exposed in utero to DEHP with supraphysiological concentrations of hCG or ACTH resulted in steroid levels similar to that of hormone-stimulated control male offspring. Taken together, these data suggest that the endocrine-disrupting effects of in utero exposure to DEHP in the testes and adrenals can be reversed by treatment with supraphysiological concentrations of the corresponding pituitary hormone mediators. Moreover, in agreement with our previous observations using the zona glomerulosa of the elderly rat, the present results indicate that the deleterious effects of in utero DEHP exposure on testis and adrenal steroidogenesis can be reversed by pharmacological means.
Funding: Supported by CIHR grant MOP-111131 and a CRC.

20

PATERNAL STRESS REPROGRAMS EPIGENETIC MARKS IN SPERM AND EPIDIDYMIS: IMPLICATIONS FOR LONG-TERM TRANSMISSION OF EXPERIENCE

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University of Pennsylvania
(Presented By: Jennifer C. Chan, BA)

Introduction: Epidemiological studies suggest that epigenetic inheritance of paternal experiences can influence disease risk in subsequent generations. Rodent models, where males do not participate in offspring rearing, provide an exciting model for examining the unique epigenetic germ cell contribution to offspring development. Recent studies have shown that paternal exposure to various perturbations can impact offspring behavior and physiology, and have identified changes in histone modifications, DNA methylation, and/or microRNA (miR) populations as potential mechanisms of transmission.
Methods: We have developed a paternal stress model in which offspring show dramatic changes in the programming of their hypothalamus, including a significantly altered stress response as adults. Mechanistically, sperm analyses identified a significant increase in 9 miRs following paternal stress exposure. Zygote microinjection of these miRs recapitulated the offspring stress phenotype, providing a functional role for sperm miRs. However, the mechanisms by which environmental perturbations alter sperm epigenetic marks and how long these changes last are not known. Preliminary data from our model reveal that males bred three months following stress exposure still produce offspring with altered stress reactivity, suggesting lasting effects of experience on intergenerational transmission. Our current studies examine the mechanism by which chronic stress alters sperm miR content through reprogramming of the male reproductive tract. Sires exposed to chronic stress were bred one week or three months following stress end to examine the rapid and long-term impact of paternal stress on the germline and offspring stress reactivity. To determine where in the sperm development or maturation process stress-mediated changes occur, we compared miR expression in the testes and epididymis.
Results: Transcriptional assessment revealed upregulation of the same stress-sensitive miRs found in sperm in the caput epididymis, but not in the testes or caudal epididymis, supporting our hypothesis that the caput is involved in programming of sperm miRs. Mass spectrometry of the caput also identified changes in histone post-translational modifications as a potential epigenetic mechanism by which stress can alter the germline. These studies suggest that paternal experiences can have lasting changes on the germline and future offspring, and offer an exciting mechanism by which the environment can dynamically regulate sperm epigenetic marks.

21

EFFECT OF PRENATAL EXPOSURE TO 1,2-CYCLOHEXANE DICARBOXYLIC ACID DIISONONYL ESTER (DINCH) ON OFFSPRING TESTICULAR FUNCTION

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(Presented By: Enrico Campioli, PharmD, PhD)

Introduction: 1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) is a plasticizer introduced in 2002 in the European market for use in plastic materials and articles intended to come into contact with food. Although DINCH received final approval from the European Food Safety Authority in 2006, there is limited knowledge about its potential endocrine-disrupting properties. Bisphenol A, a chemical used as intermediate in polycarbonate plastic and epoxy resin synthesis, and phthalate plasticizers, have been associated with the development of endocrine and reproductive diseases and different types of cancer.
Methods: Recent studies in our laboratory showed that one of the metabolites of DINCH, 1,2-dicarboxylic acid mono isononyl ester (MINCH), was able to promote in vitro rat preadipocyte differentiation through the peroxisome proliferator-activated receptor (PPAR)-α pathway in a manner similar to the phthalate metabolite MEHP. The purpose of the present work was to determine whether in utero exposure to DINCH could affect testis function in adult male Sprague-Dawley rats exposed in utero to 1, 10 and 100 mg DINCH/kg/day from gestational day 14 until birth.
Results: In utero exposure to DINCH did not affect body weight and anogenital distance of the male offspring at PND 3, 6, 21 and 60. Gene markers of somatic and germ cell function in the testis, including steroid production and androgenic activity, such as Ar, Insl3, Nes, Oct4, Lhcgr, and Star were increased at PND 3 and 6, suggesting a potential perturbation of neonatal testis function in response to DINCH exposure. In contrast, in PND 60 animals, the opposite effects were observed; e.g. Ar levels were significantly reduced starting at 10 mg DINCH/kg/day. Moreover, DINCH exposure resulted in a dose-dependent reduction of serum testosterone levels and seminal vesicle weight. Taken together, these results suggest that exposure to DINCH during a fetal period critical for testicular development induces changes in the endocrine and reproductive system of the male rat offspring.
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ABSTRACTS

22

NEGATIVE EFFECTS OF MARIJUANA (CANNABIS SATIVA) CONSUMPTION IN MALE FERTILITY POTENTIAL: TESTIS VOLUME, SEXUAL HORMONE LEVELS AND SEMINAL PARAMETERS EVALUATION

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(Presented By: Juliana Risso Pariz, BSc, MSc)

Introduction: Marijuana is the most consumed drug in the world, with approximately 200 million users. Studies in humans and animals denote that the psychoactive agent, delta-9-tetrahydrocannabiol (THC), affects hypothalamic-pituitary-gonadal axis reducing hormones released by the pituitary gland and affecting germ cells and Leydig cells. The aim of study is to evaluate the effects of Marijuana consumption in male fertility potential expressed by testis volume, hormonal levels and semen analysis.
Methods: The Universidade de São Paulo Ethics Committee approved this prospective study. We studied fifty male subjects aged 21-50 years-old (mean 32.37±7.35) who came for an initial andrological evaluation and who self-reported use of marijuana. These subjects were paired by age and compared with fertile men (pre-vasectomy candidates or no risk factors for sperm/testicular dysfunction) by Paired-Samples T Test (p adopted <0.05). Were evaluated seminal parameters (World Health Organization criteria), morphology by strict criteria, radical oxygen species levels, hormonal profile and testis volume (orchidometer).
Results: The subjects consumed Marijuana ±17.09 times a month by approximately 159.82 months. Significant negative effects of Marijuana in seminal parameters was found by alterations in pH (8.17 ± 0.36 vs. 7.68 ± 0.36; p<0.001), total motility (55.08 ± 22.64 vs. 66.02 ± 8.92%; p=0.008), progressive motility (34.48 ± 21.87 vs. 44.34 ± 10.76%; p=0.02) and morphology by WHO (12.0 ± 11.25 vs. 21.16 ± 9.45%; p<0.001) and strict criteria (2.29 ± 3.14 vs. 6.26 ± 4.30%; p=0.001) was observed a frequency of 24% of azoospermia. The level of ROS was 17.81 ± 19.67 vs. 0.22 ± 0.22 x104 com/106 sperm of control group (p=0.079). Total testosterone, LH and FSH levels not showed significant difference, however, the prolactin level of marijuana consumers increased when compared with control patients (9.0 ± 5.05 vs. 5.25 ± 2.17 µg/L; p=0.001). Finally, testis volume in marijuana group was 14.42 mL (left) and 16.15 mL (right) (vs. 21.7mL; p=0.001).
Conclusion: Our results suggest that marijuana consumption has severe deleterious effects on male reproductive health in (I) pre-testicular function, by increasing prolactin level, (II) testicular function, by decreasing testis volume, affecting spermatogenesis and reducing the morphology quality, and (III) post-testicular function, by pH alteration and reducing of sperm motility.

23

BODY MASS INDEX OR BODY FAT PERCENTAGE? AN INITIAL REPRODUCTIVE OVERVIEW FOR TWO OBESITY CLASSIFICATIONS

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(Presented By: Caroline Ranéa)

Introduction: Due to the rapid increase in development of obesity in world population, the interaction between obesity and male fertility has been widely discussed, as excess weight men have reduced reproductive potential with hormonal and seminal changes. Aim: To evaluate the effect of body mass index (BMI) and body fat percentage (BFP) in semen quality.
Methods: In this study, we used data from medical records of 83 infertile patients between 21 and 45 years ranked from the bioelectrical impedance values according to BMI [eutrophic (n=34; 18.5<BMI≤24.9 kg/m2), overweight (n=31; 25.0 <BMI≤29.9 kg/m2) and obese (n=18; BMI≥30 kg/m2)]; and BFP (n=27, normal range≤19%; n=56, high≥19%). The patients underwent semen analysis, evaluation of anti-sperm antibodies (ASA), creatine kinase activity as maturity marker (CK) and hormonal profile (testosterone, prolactin, estradiol, LH and FSH). Student´s T test and ANOVA were used to compare the means (p=0.05). Azoospermic patients were excluded.
Results: When classified by BMI, motility grade A diminished in overweight groups (1.33 ± 1.2%) and obesity (1.11 ± 2.74%) when compared to eutrophic group (3.38 ± 4.72 %; p = 0.05). Motility grade C increased in the overweight group (25.50 ± 10.11%) and obesity (25.28 ± 11.3%) when compared to eutrophic group (18.82 ± 10.15%; p=0.02). We observed an increase in concentration of ASA in overweight group (22.17 ± 11.42% vs. eutrophic 11.33 ± 8.18%, p=0.05). When classified according to the BFP, we observed reduction in number of progressive sperm (69.87±104.90 vs. 40.55±57.19; p=0,038) and reduced sperm maturation (0.22 ± 0,38U/l vs. 1.87 ± 4,90U/l; p = 0.007) in BFP≥ 19% group. No significant alterations in hormone levels were observed.
Conclusion: The accumulation of body fat, measured both by BMI or BFP, have negative effects on male fertility, as it alter mainly the sperm motility and sperm function parameters. Thus, we suggest that both assessments should be included in the assessment of male fertility.
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Ethics Committee Approval: Comitê de Ética da FMUSP (n°859215/2014)

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ABSTRACTS

24

A NOVEL STEM LEYDIG CELL CULTURE SYSTEM AND ITS USE IN ASSESSING THE EFFECTS OF PHTHALATE EXPOSURE ON LEYDIG CELL DEVELOPMENT

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(Presented By: Rose Schrott, BA)

Introduction: After eliminating Leydig cells in the adult testis with ethane dimethanesulfonate (EDS), a new population forms, arising from stem cells.
Methods: Using isolated rat seminiferous tubules, we showed that stem cells associated with the tubules proliferate and then differentiate in response to LH, and that specific Sertoli cell secreted factors are involved. In particular, FGF2 and PDGFBB were found to facilitate the proliferation of the stem Leydig cell population during the first week of culture, and Desert hedgehog (DHH) to commit the stem cells to a differentiation pathway during weeks 2-3. The testosterone (T)- producing Leydig cells formed on the surfaces of the tubules in this way continued to produce T for many weeks. Recognizing that the use of cultured tubules would not allow the effects of individual factors on stem cell proliferation and differentiation to be determined, cells were removed from the tubule surfaces by treating the tubules with collagenase. Isolated cells cultured for one week with culture medium containing FGF2 and PDGFBB proliferated, as measured by EdU labeling. After an additional week of culture with LH-containing fresh media, added DHH stimulated differentiation and T production. We used this system to assess the effects of phthalates on the proliferation and differentiation of stem Leydig cells. The effect of phthalate on cell proliferation was addressed by incubating cells for one week with LH alone (10ng/ml), or with LH plus MEHP of increasing dose (0.01μM, 0.1μM or 1.0μM), and then incubating the cells with 2μM DHH to examine their ability to produce T. Increases in T were seen in response to MEHP exposure during week 1, with greatest increase seen at the lower concentrations. The differences in T production were consistent with numbers of cells formed during week 1, as shown by EdU labeling. To examine phthalate effects on cell differentiation, the cells were incubated in LH (10 ng/ml) for week one, and then during week 2 with LH alone or LH plus MEHP of increasing dose, as above, and finally with 2μM DHH.
Results: Phthalate exposure during the second week had inhibitory effects on T production compared to LH-alone controls, with the higher doses more inhibitory than lower doses. These studies indicate that the novel system developed for examining stem Leydig cell function should be useful in studying the effects of environmental toxicants on Leydig cell development and function.

25

DRUG TOXICITY EFFECTS ON HUMAN TESTIS FUNCTIONS: 2D VERSUS 3D IN VITRO CULTURE

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(Presented By: Samuel Pendergraft, MS)

Introduction: Existing methods for evaluating potential gonadotoxicity of environmental agents and pharmaceutical compounds rely heavily on the use of animal data. However, current gold standard in vivo functional assays in animals are limited in their human predictive capacity, and existing human in vitro models of testicular toxicity do not maintain the germ cell niche of the native testis. Therefore, more reliable testicular in vitro model systems will be necessary for use in initial drug screening applications in order to reduce the number of false positive responses to potential gonadotoxic drugs prior to progression into animal studies. The overall goal of this study is to evaluate a previously developed 3-dimensional (3D) human testis organoid culture system for use as both a novel predictive first tier drug-screening tool and as a model of human testicular function in vitro.
Methods: Characterization and model system evaluation of human testicular organoids was performed using live cell imaging, IF, IHC, ELISA, qPCR, TUNEL, viability, and dose-response assays.
Results: Multicellular human testicular organoids comprising of spermatogonial stem cells (SSC), Sertoli cells, and Leydig cells were evaluated as a novel screening tool for reproductive toxicity via exposure to four clinically relevant antimitotic chemotherapeutic drugs, including busulfan, cisplatin, doxorubicin and etoposide. Organoids under all drug treatment conditions maintained IC50 values significantly higher than cells cultured in 2D. A significant decrease in apoptosis was observed in 3D organoid cultures when compared to 2D for three of the four drug treatment conditions, and specific genes expression analysis for spematogonia, sertoli and leydig cells following drug treatment further revealed that expression of cell-specific markers maintained significantly higher in 3D cultures.
Conclusions: Our 3D organoid showed significant advantages to compare with traditional 2D cultures for determinants including viability, apoptosis, morphology, and gene expression. Thus, this system has the potential to be optimized for use as a novel testicular toxicity-screening tool. Future directions include functional analysis and optimization of organoid spermatogenic capacity.
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26

PHTHALATE EXPOSURE AND SPERM DNA METHYLATION AMONG MEN SEEKING IVF TREATMENT

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(Presented By: Haotian Wu)

Introduction: Phthalates, a class of endocrine disrupting compounds, are pervasive in commercial products and recent data suggest continued and extensive human exposure to phthalates. Male germ cells require precisely coordinated epigenetic reprogramming events during spermatogenesis. An emerging body of evidence suggests that environmental exposures during this critical window of susceptibility may alter the methylome and affect reproductive outcomes and the trajectory of offspring health. In particular, epidemiologic data have previously associated phthalate exposure to negative male reproductive outcomes and offspring neurodevelopment. It is possible that these effects are in part mediated by DNA methylation.
Objectives: Examine the association between urinary phthalate metabolites and sperm DNA methylation.
Methods: A total of 48 male participants were recruited from the Baystate Medical Center's IVF clinic from 2014 to 2015 as part of the Sperm Environmental Epigenetics and Development Study (SEEDS). Seventeen urinary phthalate metabolites were analyzed from spot urine samples via LCMS by the CDC. DNA methylation was assessed with the HumanMethylation 450K array. The ChAMP (v1.8.0) pipeline was used to remove unreliable probes and correct for batch effects. GEE models were constructed with packages Aclust (v2.0.1) and geepack (v1.2.0) in R (v3.2.2). Benjamin Hochberg method was used to correct for multiple comparisons.
Results: A total of 359,968 CpG sites remained for analysis after removing unreliable probes and were subsequently allocated to 5298 clusters with a mean of 3.32 CpG sites per cluster. After adjusting for urinary specific gravity, each urinary phthalate was significantly associated with multiple methylation clusters. No common significant clusters were found among high (MECPP, MEHHP, MEOHP, MEHP and MBzP) or low (MBP, MEP, MiBP) molecular weight urinary phthalate metabolites, but many were found among metabolites of the same parent compound.
Conclusion: To our knowledge, this is the first study to present associations between phthalate exposure and sperm DNA methylation in humans. These results suggest that paternal environmental exposure encountered in adulthood may influence epigenetic reprogramming during spermatogenesis. Future analyses will investigate the biological relevant of affected pathways, using ontological analyses, and whether phthalate-associated sperm DNA methylation patterns are linked to early life development.

27

THE BINDER OF SPERM HOMOLOG 2 (BSPH2) GENE KNOCKOUT BY CRISPR/CAS9 SYSTEM AND THE EFFECT ON IN VIVO FERTILITY

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(Presented By: Marzieh Eskandari Shahraki, PhD student)

Introduction: Sperm capacitation is crucial for the encounter and fusion of gametes. During capacitation important changes on the sperm membrane are mediated by a family of proteins, ubiquitous among mammals, termed Binder of SPerm (BSP). These BSP proteins are expressed exclusively in the male reproductive tract where they bind choline phospholipids on sperm membranes and promote capacitation. Our recent studies have shown that one BSP family member BSPH1 is expressed in human epididymides whereas two are expressed in murine epididymides, BSPH1 and BSPH2.
Objective: Elucidate the functions of BSP proteins in fertilization in vivo.
Methods: Single (either Bsph1-/- or Bsph2-/-) and double (Bsph1-/-and Bsph2-/-) knockout mice were constructed using the revolutionary new targeted genome editing technique of Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated nuclease 9 (CRISPR/Cas9).
Results: The results from Bsph2-/- mice are presented here. Progeny carrying mutated Bsph2 alleles were screened by PCR as well as qPCR, and the deficit in BSPH2 mRNA expression was confirmed by RT-PCR. The preliminary data suggest that male Bsph2-/- mice are fertile in terms of average pup weight, average litter size, and number of delivery. Further studies will be carried out to evaluate sperm functions (motility, viability, capacitation, acrosome reaction), and reproductive organ morphology. We also plan to characterize Bsph1-/- mice as above.
Conclusion: Moreover since Bsph1 and Bsph2 may exert overlapping function(s), it will be important to characterize the double knockout as well. (Supported by CIHR, and the FESP of the University of Montreal).

28

ANDROGENS ARE ESSENTIAL FOR EPITHELIAL CELL RECOVERY AFTER EFFERENT DUCT LIGATION (EDL) IN THE PRE-PUBERTAL EPIDIDYMIS

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(Presented By: Bongki Kim, PhD)

Introduction: Deprivation of testicular luminal factors by EDL induces a dramatic degeneration in epithelial cells of the epididymal initial segment (IS), which is followed by regeneration. Little is known on the mechanisms involved during the regeneration and repair phase that follows EDL. We tested here the hypothesis that exposure of androgens during postnatal development is necessary for acquisition of the recovery ability of the regressed epithelium after EDL.
Methods: Unilateral EDL was performed to block luminal flow from the testis without affecting blood flow in 3-, 4-, 5-, 6- and

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7-week old mice. Cell-specific epithelial cell proliferation was examined 2 and 2.5 days after EDL by immunofluorescence for Ki67. Triple labeling was performed for Krt5, a marker of basal cells (BCs) and the V-ATPase (B1 subunit), a marker of narrow cells (NCs). Principal cells (PCs) were identified by negative selection (Krt5(-) and V-ATPase(-)).

Results: A progressive increase in the number of proliferating BCs was observed 2 days post-EDL at postnatal week (PNW) 5, 6 and 7 (Ctrl: 0.95 vs EDL: 8.92/tubule, $p<0.001$ at PNW7), while the number of proliferating PCs still remained low. At 2.5 days post-EDL, the number of proliferating BCs was reduced to 3.01/tubule at PNW7, while the number of proliferating PCs increased (Ctrl: 1.59 vs EDL: 11.16/tubule, $p<0.001$). No increase of proliferation was seen in NCs. These results indicate different time courses for regeneration of BCs (2 days after EDL) and PCs (2.5 days after EDL) after epithelial regression by EDL. In 3- and 4-week old mice, however, no increase in proliferation rates was seen in BCs and PCs, despite the induction of apoptosis (examined using caspase 3) by EDL. This finding indicates that the ability of the epithelium to repair itself after damage strongly depends on the age of the mice. We examined whether elevation of androgens during the peripubertal period plays a role in epithelial recovery. 4-week old mice were treated with flutamide for 21 days, and EDL was performed with continuous infusion of flutamide for an additional 2 or 2.5 days. Flutamide treatment dramatically decreased the number of proliferating BCs (Ctrl: 8.92 vs Flu: 1.91/tubule, $p<0.001$) on day 2 post-EDL, and the number of proliferating PCs (Ctrl: 11.16 vs Flu: 0.05/tubule, $p<0.001$) on day 2.5 post-EDL, compared to the control group. This study indicates that androgen stimulation during postnatal epididymal development plays a key role in epithelial repair after injury.

29

REGULATION OF LUMINAL ACIDIFICATION BY PRINCIPAL AND CLEAR CELLS IN THE MOUSE EPIDIDYMIS

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(Presented By: Yoojin Park, PhD)

Introduction: Clear cells (CCs) and principal cells (PCs) are specialized epithelial cells that regulate luminal acidification in the cauda epididymidis (Cd), a process that is essential for the maturation of spermatozoa. CCs express the proton-pumping ATPase (V-ATPase) in their apical membrane and are major contributors to H+ secretion. In addition, PCs secrete H+ via the sodium/hydrogen exchanger (NHE3) also present in their apical membrane. We examined here the regulation of luminal acidification by CCs and PCs, via the V-ATPase and NHE3, respectively.

Methods: The mouse Cd was perfused with a low-buffered solution adjusted to the control pH 6.6, and the alkaline pH 7.8, in the absence or presence of the cpt-cAMP (1mM). The perfusate was collected via an incision made at the epididymal/vas deferens junction, and the pH was measured immediately using a miniature microelectrode.

Results: The pH of the control solution remained constant at 6.6 after its passage through the Cd. The pH 7.8 perfusate progressively decreased from 7.8 to 7.12 ± 0.10 after 10 min and 7.13 ± 0.11 after 20 min. The V-ATPase inhibitor, concanamycin A, and the NHE inhibitor, EIPA, each induced partial inhibition of luminal recovery, while total inhibition was induced when they were applied together. These results show the participation of CCs and PCs in luminal acidification. Addition of 1 mM cpt-cAMP at pH 7.8 slowed down luminal pH recovery from 7.8 to 7.39 ± 0.02 after 10 min and 7.13 ± 0.11 after 20 min, indicating partial inhibition of H+ secretion by cAMP. In other organs, NHE3 is inhibited by cAMP, via internalization from the plasma membrane. Cell fractionation studies showed that NHE3 is redistributed from intracellular vesicles to the plasma membrane when the Cd was perfused at pH 7.8 compared to 6.6, indicating that it is regulated via recycling in the epididymis. In addition, cpt-cAMP prevented the alkaline pH induced NHE3 redistribution. In addition, a significant redistribution of the V-ATPase from intracellular vesicles to the plasma membrane was observed in the Cd perfused at pH 7.8 + cpt-cAMP compared to pH 6.6.

Conclusion: Our results: 1) suggest that cAMP inhibits NHE3-dependent H+ secretion via NHE3 redistribution from the plasma membrane to vesicles; and 2) confirm our previous results showing that cpt-cAMP induces the accumulation of V-ATPase in the plasma membrane. Therefore, our study indicates the complex role of cAMP in the regulation of luminal acidification by CCs and PCs in the cauda epididymidis.

30

ROLE OF DICER1-DEPENDENT MICRORNAS IN THE PARACRINE CONTROL OF EPIDIDYMAL GENE EXPRESSION

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(Presented By: Olivia Jerczynski)

Introduction: Dicer1 is an endoribonuclease involved in the biogenesis of functional microRNA (miRNA). These small non-coding RNA are important regulators of the post-transcriptional gene expression and participate to the control of male fertility. Knowing that (1) Dicer-dependent factors are needed for proper sperm maturation in the epididymis, and that (2) miRNAs are potent mediators of intercellular communication in most biological systems, we investigated the role of Dicer dependent-miRNAs produced from the proximal epididymis (initial segment/caput) on the paracrine regulation of epididymal gene expression in the distal epididymis regions (i.e. corpus and caudal) (Figure 1).

Methods: To this aim, we performed comparative microarray and ANOVA analyses on control vs. Defb41-iCre; Dicer1fl/fl mice, in which functional Dicer1 is absent from the principal cells of the proximal epididymis. We identified 21 and 16 transcripts, including Prostate And Testis Expressed 4 protein and the Zn-alpha 2-glycoprotein, that display significant expression level changes in the corpus and cauda regions, respectively (Fold change >2 or <-2; $p<0.001$). In addition, 154 miRNAs, including miR-210, miR-672, mi-R-191 and miR-204, show a significantly impaired biogenesis in the absence of Dicer1 from the proximal

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epididymis (Fold change>2 or <-2; p<0.01). These miRNA are secreted via EVs derived from DC2 epididymal principal cell line, and their expression correlates with target transcripts involved in important biological pathways, as evidenced by in silico analysis.
Results: This suggests that Dicer-dependent miRNAs could act as potent paracrine regulators of epididymal functions (i.e. control of sperm motility, ciliogenesis) and may contribute to the infertility phenotype observed in the Defb41-iCre; Dicer1fl/fl mouse model. These findings open new avenues for the identification of molecular targets important to male fertility control.
Funding: Sponsored by a NSERC grant to C. Belleannée and Centre de Recherche en Développement et Santé Intergénérationnelle (CRDSI) Scholarship to O. Jerczynski.

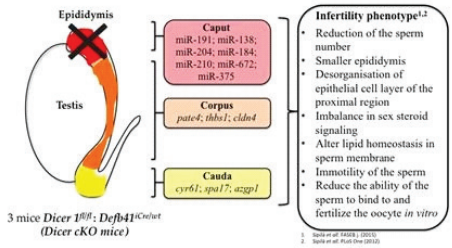

Figure 1: Effect of the Dicer enzyme inactivation in the head of the epididymis on production of microRNAs and the expression of downstream genes.

31

INTERLEUKIN 6 INHIBITS THE DIFFERENTIATION OF RAT STEM LEYDIG CELLS INTO LEYDIG CELL LINEAGE

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(Presented By: Yiyan Wang)

Introduction: Stem Leydig cells are present on the surface of seminiferous tubules in the testis. The factors regulating the differentiation of rat stem Leydig cells into Leydig cell lineage are largely unknown. The objective of the present study was to investigate whether interleukin 6 (IL6) affects the differentiation of stem Leydig cells.
Methods: Adult male Sprague Dawley rats were i.p. injected 75 mg/kg of ethane dimethane sulfonate (EDS). Four days after EDS injection, testis was collected and detached as single seminiferous tubules that were cut by 0.25 inch long.
Results: There were no Leydig cells on the surface of the seminiferous tubules upon isolation, suggesting that all Leydig cells were eliminated by EDS. The seminiferous tubules were cultured in M199 and DMEM/F12 medium at CO2 5%, 37C for 14 days. Medium testosterone levels were below detectable level during first 7 days of culture. We found that luteinizing hormone (LH) or insulin-transferrin-selenium failed to induce rat stem Leydig cells to differentiate into Leydig cell lineage when they were used alone while the combination of them significantly induced the stem Leydig cells into Leydig cell lineage with robust testosterone production with the increase of testosterone production over 150%. Lithium chloride (LiCl), a GSK-3b agonist, significantly promoted the differentiation of the stem Leydig cells with the increase of testosterone level over 200% when compared to basal medium or 120% over LH+ITS group, and further study demonstrated that lithium actually exerted it action at the stage of the progenitor into immature Leydig cells. At 14 days after culture, the induced Leydig cells on the surface of seminiferous tubules robustly produced testosterone, with the level over 1000 pg/ml. Using the appropriate Leydig cell differentiation medium that contains LH (5 ng/ml), ITS (10 μg/ml), and LiCl (5 mM) together with various concentrations of IL6 (1, 10, 100ng/ml) for 14 days. IL6 concentration-dependently decreased testosterone production, with 70 folds lower in 100 ng/ml concentration. QPCR showed Leydig cell biomarker, Cyp11a1, Cyp17a1, Hsd17b3, and Lhcgr were concentration-dependently decreased, while the biomarker Hsd3b1 and Hsd11b1 were unchanged. These results demonstrate that IL6 is an inhibitory factor for differentiation of stem cells. Furthermore, we also tested the effects of IL6 on stem Leydig cells using EDU-incorporation and we found that IL6 promoted the proliferation of stem Leydig cells.

32

EFFECTS OF IN UTERO EXPOSURE OF TETRABUTYLTIN ON FETAL LEYDIG CELLS

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(Presented By: Yaoyao Dong, Sr., master)

Introduction: Tetrabutyltin is a stable organotin. It is the starting material for a wide range of organotin compounds used as stabilizers for polyvinyl chloride plastics, biocides, fungicides and anti-biofouling agents. It has been found to be a potential endocrine disruptor. The objective of the present study is to investigate effects of tetrabutyltin on fetal Leydig cell function.
Methods: Female pregnant Sprague Dawley dams orally received vehicle (corn oil, control) or tetrabutyltin (100, 200, and 500 mg/kg) from gestational day (GD) 12 to GD 21. At GD 21.5 (postnatal day 1), serum testosterone production, fetal Leydig cell numbers and testicular gene expression levels were examined.
Results: Tetrabutyltin showed dose-dependent reduction of serum testosterone levels (0.756 ± 0.208 and 0.813 ± 0.277 ng/ml for 200 and 500 mg/kg, respectively) when compared to the control (1. 692 ± 0.218 ng/ml). QPCR showed that Scarb1, Cyp17a1, and Insl3 expressions were downregulated at 100 mg/kg and Star levels at 500 mg/kg. At higher dose (500 mg/kg), tetrabutyltin also reduced Leydig cell number. The results indicate that in utero exposure to tetrabutyltin affects the expression levels of some fetal Leydig cell steroidogenic genes and fetal Leydig cell number.

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33

EFFECT OF PROPOFOL ON THE STEROIDOGENESIS OF RAT IMMATURE LEYDIG CELLS

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(Presented By: Xiaolong Wu, Sr., master)

Propofol is an anesthetic. Whether propofol disrupts Leydig cell steroidogenesis is unclear. When rat Leydig cells were exposed to propofol, 100 μM propofol significantly inhibited luteinizing hormone and 8br-cAMP stimulated androgen production. At this concentration, it also inhibited 22R-OH-cholesterol, pregnenolone-mediated androgen production, suggesting that it inhibits these testosterone biosynthetic enzymes, cholesterol side chain cleavage enzyme (CYP11A1) and 3β-hydroxysteroid dehydrogenase (3β-HSD). However, it did not inhibit progesterone and androstenedione-mediated androgen production, suggesting that 17α-hydroxylase/20-lyase (CYP17A1) and 17beta-hydroxysteroid dehydrogenase 3 are not affected. Further investigation showed that propofol specifically and directly inhibited testis 3β-HSD. The half maximal inhibitory concentration (IC50) for propofol inhibition of 3β-HSD was 14.37± 0.04 μM (rat) and 20.75± 0.06 μM (human). The mode of action of propofol on 3β-HSD activity was competitive with the substrate pregnenolone, but was mixed with the cofactor NAD+. At the higher concentration of 100 μM, propofol did not affect human and rat testis CYP17A1 and 17β-hydroxysteroid dehydrogenase 3 activities. The inhibition of testis 3β-HSD by propofol was more specific, since propofol only weakly inhibited another 3β-HSD isoform (3β-HSD1) in the human placenta. In summary, our results showed that propofol has the capacity for direct inhibition of 3β-HSD activity. Inhibition of testicular 3β-HSD enzyme activity is presumably associated with suppression of steroidogenesis in gonadal tissues.

34

IMMATURE SERTOLI CELLS HAS HIGHER EXPRESSION OF 17?-HYDROXYSTEROID DEHYDROGENASE 3 THAN FETAL LEYDIG CELLS IN NEONATAL RAT TESTIS

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(Presented By: Baiping Mao, Sr., master's degree)

Introduction: Testosterone is a primary androgen in the fetal and neonatal males. In rat fetal testis, the fetal Leydig cells are thought to be the endocrine cells. Whether Sertoli cells are required for the production of testosterone in the fetal/neonatal rat testis is not fully unclear.
Methods: In the present study, we used a specific fetal Leydig cell eliminating agent, ethane dimethane sulfonate (EDS), to kill fetal Leydig cells in the neonatal testis to investigate the expression of 17beta-hydroxysteroid dehydrogenase 3 (HSD17B3). Real-time PCR (qPCR) was used to detect the expression level of Hsd17b3.
Results: Hsd17b3 was higher at neonatal testis at postnatal day 1, dramatically declined to undetectable between postnatal days 14 and 21 and resumed its expression at postnatal day 28 and reached the highest level at postnatal day 56. 75 mg/kg EDS was administered intraperitoneally to male pups at postnatal day 3. One day after EDS (postnatal day 4), fetal Leydig cells were completely eliminated as evidence that undetectable level of Leydig cell specific gene 3beta-hydroxysteroid dehydrogenase 1 (Hsd3b1) expression, undetectable serum testosterone level, and no fetal Leydig cells existing in the interstitium were observed. The second population of Leydig cells, the adult Leydig cells, appeared four days after EDS (postnatal day 7) and Hsd3b1 expression levels reached the highest levels between 10 and 17 days after EDS (postnatal days 14 and 21). Immunohistochemical staining showed the abundant expression level of Hsd17b3 in immature Sertoli cells and low level in fetal Leydig cells at postnatal day 1. Using magnetic bead with anti-luteinizing receptor antibody to purify fetal Leydig cells at postnatal day 7 and adult Leydig cells at postnatal day 56 and compare the expression of Hsd17b3, we found that Hsd17b3 expression level was detectable but three folds lower than that in adult Leydig cells and 7 folds lower than that in immature Sertoli cells from postnatal day 7. Our results revealed that the fetal Leydig cells synthesize 80% androstenedione and 20% testosterone because they had lower expression of Hsd17b3, and immature Sertoli cells fulfilled the major part of conversion of androstenedione to testosterone. However, in adult testis, adult Leydig cells synthesize testosterone because there is no expression of Hsd17b3 in mature Sertoli cells. In conclusion, fetal Leydig and immature Sertoli cells are required for testosterone biosynthesis in the rat fetal and neonatal testis.

35

ANDROGEN-RECEPTOR EXPRESSION AND LOCALIZATION IN MUSSEL SPERM

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(Presented By: Jeremy Feng)

Introduction: NY Long Island pesticides spray used to kill young mosquitos, it may impact specie's reproduction, consequently reduce species' population, and seriously impact lobster and other seafood species. The hypothesis in this study is that pesticides' toxic harm on lobster population may be through reproductive system, damaging specie's eggs and sperm function, and impacting fertilization, consequently jeopardizing species' life cycle and declining population.
Objective: In this study, the mussels were used as specie model, and its sperm was stained for the expression and localization of androgen receptor.
Methods: The mussel's testicular tissues were dissected by syringe-needle in culture medium (HTF plus 10% serum), the sperm was released into culture medium, soaked supernatant tissue liquid to glass slide and dried by air, then fixed by methanol and canoy's solution (methanol: glacial acid 3:1) for 15 minutes, then performed immunochemical-staining with anti-androgen antibody. Then after, the slides will be stained by

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Zymed kit following manufacture instruction. The slides will be observed under computerized compound microscope.
Results: In the present study, it was firstly demonstrated the presence of a functional AR in mussel sperm. AR immunoreactivity was specifically compartmentalized at the sperm head. No immunoreaction was detected either by adding anti-AR antibody peptide by immuno precipitation or when the primary antibody was omitted.
Conclusion: AR immunoreactivity was specifically compartmentalized at the sperm head, which may play important role in mussel life cycle. * Internship student. 1. zhuw@oldwestbury.edu

37

CHES-1-LIKE, THE ORTHOLOG OF A NON-OBSTRUCTIVE AZOOSPERMIA ASSOCIATED GENE, BLOCKS GERMLINE STEM CELL DIFFERENTIATION THROUGH UPREGULATING DPP EXPRESSION IN THE DROSOPHILA TESTIS.

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(Presented By: Jun Yu, PhD)

Azoospermia is a high risk factor of testicular germ cell tumor, the most common cancer in young men, but the underling molecular mechanisms are not clear. In a genome-wide association study trying to identify novel loci associated with human non-obstructive azoospermia (NOA), we uncovered a single nucleotide polymorphism (rs1887102, P=2.60X10-7) in a human gene FOXN3. FOXN3 is an evolutionary conserved gene. We further used Drosophila as a model system to test whether CHES-1-like, the FOXN3 otholog, is required for male fertility. The CHES-1-like knockout flies are viable and fertile and show no defects in spermatogenesis. However, ectopic expression of CHES-1-like in germ cells significantly reduced male fertility. With CHES-1-like overexpressing, the spermatogonia fail to cease mitotic division after four rounds but continue to divide to form tumor like structures. In these testes, the expression levels of differentiation factor Bam were reduced but the expression region of Bam was expanded. Further reduce Bam expression in the CHES-1-like expressing testes enhanced tumor like structure formation. The expression of daughters against dpp (dad), a downstream gene of dpp signaling, was upregulated by Ches-1-like expression in testes. We found that overexpressing Ches-1-like could upregulate dpp expression in wing imaginal discs. In testis, CHES-1-like could directly bind to dpp promoter. We proposed a model that CHES-1-like overexpression in germ cells could upregulate dpp expression and inhibit germ cell differentiation and finally lead to germ cell tumors.

38

LEUKEMIA INHIBITORY FACTOR STIMULATES STEROIDOGENESIS OF RAT IMMATURE LEYDIG CELLS VIA INCREASING THE EXPRESSION OF STEROIDOGENIC ACUTE REGULATORY PROTEIN

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(Presented By: Xiao-Heng Li, MS)

Introduction: Leukemia inhibitory factor (LIF) is a glycosylated cytokine and has many physiological roles. However, its effects on Leydig cell development are still unclear.
Methods: Immature and adult Leydig cells isolated from 35 and 90-day-old rats were cultured with different concentrations of LIF alone or in combination with LH for 24 hours. Then cells were further cultured without or with LH and 8Br-cAMP, for 3 hours. The concentrations of testosterone and 5α-androstanediol in the media were measured by radioimmunoassay. Q-PCR was used to measure the mRNA levels of following genes: Lhcgr, Scarb1, Star, Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b3, Srd5a1, Akr1c14, Ccnd1 and Insl3.
Results: LIF (1 and 10 ng/ml) significantly increased androgen production in immature Leydig cells when cells were cultured with LIF only. However, LIF did affect androgen production when cells were cultured with a 10 ng/ml of LH. LIF alone or in combination with LH had no effects on testosterone production in rat adult Leydig cells. Further studies revealed that LIF dose-dependently increased Star expression level in immature Leydig cells. However, when in combination with LH, the compromise of Lhcgr may explain the ineffectiveness of LIF in LH-stimulated androgen production. Gene microarray revealed that the upregulation of anti-oxidative genes and Star might contribute to LIF-induced androgen production. In conclusion, LIF has stimulatory effects on androgen production in rat immature Leydig cells.

39

CALCIUM IONOPHORE A23187 INDUCES IN-VITRO FERTILIZATION CAPACITY IN SPERM FROM STERILE KNOCKOUT MODELS

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(Presented By: Felipe A. Navarrete, BS)

Introduction: Mammalian sperm acquire fertilizing capacity in the female tract in a process called capacitation. As part of capacitation, sperm undergo changes in their motility pattern (i.e., become hyperactivated) and become prepared for an exocytotic acrosome reaction, which is necessary for fertilization. At the molecular level, capacitation requires a fast activation of protein kinase A (PKA) which is followed by hyperpolarization of the sperm plasma membrane and an increase in intracellular Ca2+. Genetic or pharmacological inhibition of these pathways results in male infertility in vivo and in vitro. Recently, we demonstrated that incubating mouse sperm with the Ca2+ ionophore (A23187) for a short period accelerates capacitation and induces fertilizing

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capacity in sperm even when PKA activation is blocked. Based upon these results, we hypothesized that A23187 could be used to overcome defects in signaling pathways upstream of the increase in intracellular Ca2+ required for capacitation.
Objective: to study the effect of short pulses of intracellular Ca2+ in the fertilizing ability of different infertile knockout (KO) models.
Methods: application of Ca2+ ionophore pulse, in vitro capacitation, in vitro fertilization, embryo transfer, and computer assisted sperm analysis (CASA) were performed.
Results: We found that a pulse of ionophore before capacitation induces hyperactivated motility and fertilization of sterile CatSper1 (sperm specific Ca2+ channel), Adcy10 (soluble adenylyl cyclase sAC) and SLO3 (sperm-specific K+ channel) KO mice. Moreover, embryos obtained from in vitro fertilization of Catsper1 KO were transferred to pseudopregnant females and give birth live pups. In contrast, sperm from infertile mice lacking the Ca2+ pump PMACA4 were not rescued by ionophore. These results indicate that a temporary increase in intracellular Ca2+ can be used to overcome genetic infertility in mice.
Conclusion: Altogether, our results suggest that this approach overcomes genetic models of mouse infertility and may serve as a foundation to treat certain human male infertility problems.

40

ALTERNATIVE SEX HORMONE-BINDING GLOBULIN PROMOTER TRANSCRIPTIONAL ACTIVITY IN TESTICULAR GERM CELLS

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(Presented By: Warren Meyers, BSc)

Introduction: It is well established that sex hormone-binding globulin (SHBG) is a product of Sertoli cells in the rodent testis. The human SHBG gene is repressed in Sertoli cells and is instead transcribed from an alternative transcriptional start site within developing germ cells. Sperm SHBG is first visible within the proacrosomal granule of stage VII round spermatids and is retained within the acrosome of mature spermatozoa. In this study, we investigated mechanisms controlling transcription from the alternative SHBG promoter.
Methods: A transient reporter system was used to model transcriptional activity from the alternative SHBG promoter (altP-SHBG). The promoters controlling the proximal (-266/+366) and alternative (-336/+28) SHBG transcription units were cloned into the pGL3 luciferase reporter plasmid and transfected into the GC-2spd(ts) immortalized mouse spermatocyte cell line. Forty-eight hours after transfection cell lysates were harvested and assayed for luciferase reporter activity. RNA extractions, cDNA synthesis and isolation of cytoplasmic and nuclear fractions from GC-2 cells were done using conventional methods. For chromatin immunoprecipitation (ChIP) assays, total chromatin was isolated from the seminiferous tubules of our transgenic mice that harbour an 11 kb region of the human SHBG gene.
Results: The human alternative SHBG promoter is highly active in GC-2 cells ($P<0.0001$), whereas the proximal SHBG promoter fails to produce a signal above background. We identified two CACCC elements within the minimal region of the alternative SHBG promoter. Disruption of both elements results in a 75% decrease in reporter activity ($P<0.0001$). Krüppel-like factor 4 (KLF4) is known to interact with CACCC elements and is expressed in GC-2 cells. KLF4 localizes to the nucleus in these cells, consistent with its location in round spermatids in vivo. Knockdown of Klf4 results in a decrease in altP-SHBG activity ($P<0.001$) and Klf4 overexpression rescues the effects of knockdown. Finally, we show that KLF4 directly interacts with the alternative SHBG promoter in vivo using chromatin obtained from the seminiferous epithelium of our transgenic mice.
Conclusion: Our results indicate that KLF4 is required for optimal transcriptional activity from the alternative SHBG promoter. This is the first description of mechanisms that may influence SHBG expression during post-meiotic sperm development.

41

ROLE OF PATERNAL FACTORS IN EARLY EMBRYONIC DEVELOPMENT

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(Presented By: Manoj Kumar, MSc, PhD)

Introduction: Recurrent miscarriage (RM) or consecutive pregnancy loss before 20th week of gestation occurs in 1−5% women. RM usually was seen as a mother's problem, but the developing knowledge shows the role of paternal contribution for the fetal loss. However, it is still difficult to diagnose male partners who contribute to pregnancy loss with classical semen analysis. And there is need to establish other molecular diagnostic markers apart from DNA fragmentation index (DFI) which help in the best diagnosis of RM. Our objective is to study the role of sperm gene expression profile in idiopathic RM patients.
Methods: Total RNA was isolated from 30 male partners of RM couples and 30 male partners of healthy couples who have fathered healthy child and was reverse transcribed to cDNA. cDNA was amplified and quantified by qPCR and gene expression analysis was performed for TOMM7, RBM9 (RBFox2), RPS6, RPL10A, EIF5A, AKAP4, STAT4, SOX3, and FOXG1 genes.
Results: Sperm gene expression of TOMM7, RBM9, RPL10A and AKAP4 were up regulated around one fold, the genes FOXG1, SOX3 and STAT4 were up regulated more than one fold as compared to EIF5A and RPS6 in the male partners of iRM couples compared to the controls. In this study we observed increase in the gene expression of genes which are important for normal fetal development. Future studies can help elucidating their functional importance and how they regulate the fetal development and their over expression leads to embryonic lethality and recurrent miscarriage.

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ABSTRACTS

42

MICRORNA EXPRESSION FOLLOWING LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION OF RAT TESTIS

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(Presented By: Mitchell Isaac Parker)

Introduction: MicroRNAs (miRNAs) are a group of small, double-stranded, non-protein coding RNAs that negatively regulate gene expression at the transcriptional and post-transcriptional levels. Andrologists are interested in miRNAs because proper expression of these transcripts has been linked to normal testis development and spermatogenesis, while atypical expression of certain miRNAs has been implicated in testicular cancer formation and male infertility. We are interested in the functional significance of miRNAs in defending the male reproductive tract from infection.

Objective: We hypothesized that control of gene expression by miRNAs plays a significant role in the antimicrobial protection of rat testis and the response to inflammation. The overall goal of our research is to determine which miRNAs are involved in regulating inflammation of the testis in response to infection. The objective of this study was to identify inflammatory-related miRNAs (miRNAs that target inflammatory genes) that are up-regulated and/or down-regulated following lipopolysaccharide (LPS)-induced inflammation of rat testis.

Methods: A Qiagen Rat Inflammatory Response & Autoimmunity miRNA PCR Array (MIRN-105Z) was used to evaluate expression of 84 inflammatory-related miRNAs. Testis total RNA was purified from retired Sprague-Dawley breeder rats that were sacrificed three or six hours after receiving a 5 mg/kg injection of LPS (n=4) or saline (n=2), and examined by quantitative real-time polymerase chain reaction (qPCR).

Results: Results showed 5 inflammatory-related miRNAs with a greater than 2 fold down-regulation ($p<0.05$) in rats from the 3h group (let-7f-5p, miR-200c-3p, miR-28a-3p, miR-28b-3p, and miR-98-5p), and 5 inflammatory-related miRNAs with a greater than 2 fold down-regulation ($p<0.05$) in rats from the 6h group (miR-17-5p, miR-19a-3p, miR-34a-5p, miR-34c-5p, and miR-449a-5p).

Conclusions: Previous studies in our laboratory on gene expression following LPS-induced inflammation of rat testis demonstrated up-regulation of a number of inflammatory genes. Since miRNAs negatively control gene expression, we propose that the levels of certain inflammatory-related miRNAs might be decreasing upon LPS administration to increase the net number of target inflammatory genes available for translation.

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43

MAPPING GENETIC HETEROGENEITY OF VIABLE AND NON-VIABLE SPERM

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(Presented By: Liina Nagirnaja, PhD)

Introduction: The application of next-generation sequencing to male germ cells can revolutionize our understanding of germline mutation processes, and potentially lead to novel clinical assays. However, very little is known about genomic heterogeneity in normal ejaculated sperm, and how this heterogeneity may confound studies of mutation. In healthy men, approximately 70% of the sperm are found as viable; however, in infertile men the proportion of the viable sperm is absent or decreases significantly. Thus, characterizing the genetic differences between viable and non-viable sperm is an important step towards developing robust assays for mutation detection from bulk ejaculate.This study aimed to (i) assess whether MALBAC whole-genome pre-amplification is applicable for reliable exome sequencing of a small pool of sperm and to (ii) describe the genetic heterogeneity of viable and non-viable sperm by standard exome sequencing of bulk DNA.

Methods: Three DNA pools of 200 live and 200 dead sperm from a healthy male donor were amplified with MALBAC whole-genome amplification. Nextera (Illumina) exome sequencing libraries were prepared using bulk sperm DNA from blood, live and dead sperm of the same donor as well as MALBAC pre-amplified reactions.

Results: Standard exome sequencing of bulk blood and sperm DNA identified 10893 SNPs and 319 INDELs grouped as (i) de novo variants observed in sperm only or (ii) inherited variants present in blood and sperm. Germline-specific de novo variants were found to be rare in sperm as expected (n=77, mean variant allele fraction, VAF=0.13-0.23). Interestingly, inherited variants transmitted only to live or dead sperm (n=78) were mosaic in both somatic blood and sperm, with a lower frequency in sperm (from 0.26 in blood to 0.18 in dead sperm, P=0.012; from 0.18 to 0.14 in live, P=0.0004). A similar pattern was seen for INDELs transmitted to dead sperm (n=3; VAF from 0.29 to 0.13). MALBAC sequencing of 200 sperm introduced technical noise limiting the application of this method for genotyping low inputs of sperm.

Conclusion: This study identified a set of de novo and inherited variants potentially impacting the viability of developing sperm. De novo variants seen in a subset of sperm cells (13-23% based on mean variant VAF) may have occurred during spermatogenesis, whereas mosaic inherited variants likely originate from early embryonic development. Further studies are needed to elucidate the role of identified pathogenic mutations specific to live and dead sperm.

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44

HIGH DOSE FOLIC ACID SUPPLEMENTATION AND METHYLENETETRAHYDROFOLATE REDUCTASE HAPLOINSUFFICIENCY RESULT IN HYPOMETHYLATION OF THE SPERM DNA METHYLOME IN MICE

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(Presented By: Mahmoud Aarabi, MD, PhD)

Introduction: Supplementation with high doses of folic acid, an important source of methyl groups for epigenetic processes such as DNA methylation, is widely used in clinics to improve the sperm parameters of infertile men. We have recently detected alterations of DNA methylation in several important genes in the sperm of infertile men after six months of 5 mg /day (>10x the daily recommended intake) folic acid supplementation. DNA methylation changes were more prominent in men homozygous for a common variant in the gene encoding methylenetetrahydrofolate reductase (MTHFR 677C->T), an important enzyme in the folate metabolic pathway.
Methods: To address the possible mechanisms responsible for the observed epigenetic alterations in the sperm of infertile men, wildtype and heterozygote mice for a targeted mutation in the Mthfr gene (Mthfr+/-) were fed high folic acid (10x the daily recommended intake) or control diets for six months (n=6 /experimental group). Germ line-specific differentially methylated regions of the imprinted genes H19 and Snrpn were examined in sperm DNA by pyrosequencing. A next generation sequencing-based method, reduced representation bisulfite sequencing (RRBS), was utilized to study over 3 million DNA methylation sites across the sperm epigenome.
Results: Neither folic acid supplementation nor Mthfr genotype resulted in changes in DNA methylation at imprinted loci in sperm. In contrast, analysis of global sperm DNA methylation by RRBS in a subset of mice from each group revealed significant alterations of methylation in mice supplemented with high dose folic acid. Loss of methylation was the most abundant change, and was exacerbated in the sperm of Mthfr+/- mice.
Conclusion: Our findings of sperm hypomethylation associated with folic acid supplementation or lower expression of MTHFR in mice, mirror those we reported in a group of infertile men. We are now performing in-depth bioinformatics analyses as well as validation of the RRBS findings to find the most susceptible genomic regions and disease-related genes affected by alterations of DNA methylation. Unexpected loss of methylation across the sperm epigenome suggests the involvement of other factors in folate metabolic pathways. To this end, we are measuring MTHFR protein expression levels in the testes as a potential mechanism for the sperm DNA hypomethylation.
Funding: Supported by the Canadian Institutes of Health Research (CIHR).

46

HYPOASTHENOZOOSPERMIA CORRELATES WITH INCREASED DNA SPERM FRAGMENTATION

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(Presented By: Marcela Lozano Mendoza)

Objective: To evaluate the seminal parameters of infertile men evaluated at a fertility clinic in relation with sperm DNA integrity.
Design: A prospective cohort fashion, at Hospital Ángeles del Pedregal reproduction center.
Methods: A prospective longitudinal survery comprised of 250 samples of patients at ART reproduction infertility programs during 2011-2014. Conventional semen parameters, (WHO 2919 strict criteria) were measured. The DNA sperm integrity was evaluated with sperm chromatin dispersion test (SCD) using the cutoff point of 30%. Spearman tests were used to detect significant differences.
Results: We found that in 250 samples the mean age was 38 (6 ± years); the average DNA sperm integrity value was 29% (± 13.6%), in the seminal parametrs we found a a volumen of 2.6 (± 1.5 ml), pH 7.8 (± 0.5), motility 45 (± 17.2%), viability 74.1 (± 69.1%), liquefaction of 42 (± 21.3 min), morphology 16.2(± 10.2%), concentration 102.8 (± 77.6 mlls/ml). In neat semen, a positive correlation was found between the DNA sperm integrity and motility (Spearman r=-0.3 p=0.001, 95% CI), age (Spearman r=0.219 p=0.001, 95% CI). Interestingly semen volume correlated with motility (Spearman r=0.2 p=0.001, 95% CI).
Conclusions: The DNA sperm integrity evaluated with SCD correlates with sperm motility because it supports a functional sperm, but still stands out the correlation between volume and motility; it could be due to the testis micro-environment expressed in the seminal plasma. The age is also a factor that affects our population DNA sperm integrity, and it could be related to aging testosterone decrease and dysfunctional tubular sperm formation.

47

SEMEN OXIDATIVE REDUCTION POTENTIAL (ORP) IS RELATED TO ABNORMAL SEMEN PARAMETERS IN MALE FACTOR INFERTILITY

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(Presented By: Jaideep Singh Toor, PhD)

Introduction: Oxidative stress (OS) is known to impair the physiological function of spermatozoa, leading to male infertility. OS occurs due to imbalance resulting from higher production of reactive oxygen species (ROS) and lower activity of the antioxidant enzymes in semen. The assessment of such imbalance is complicated and often difficult to investigate in the clinical setting. Oxidation-reduction potential (ORP) is a newer and simpler tool to assess the status of oxidative stress. The present

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study measures both static-ORP (sORP as mVolt) and capacity-ORP (cORP current as µC) in fresh semen samples using a new electrochemical system, MiOXSYS™ to correlate ORP (as a measure of oxidative stress) with semen parameters of infertile men.
Methods: Semen samples from 24 infertile men presenting to andrology clinic were collected after informed consent. Semen analyses were first performed according to WHO guidelines. A well-mixed aliquot of 30µl was then used to measure sORP and cORP using the MiOXSYS analyzer after calibration. All values are reported as mean ± SEM and the Spearman correlation coefficient (r) was carried out using GraphPad Prism software.
Results: A significant negative correlation of sORP (mVolt/million sperm/mL) was observed with sperm concentration ($r = -0.8428$, $p<0.0001$), total sperm count ($r = -0.8043$, $p<0.0001$), sperm motility ($r = -0.3786$, $p = 0.0341$), and sperm morphology ($r = -0.4843$, $p = 0.0082$) in semen samples from infertile patients. A 7-fold decrease in mean ratio of sORP/cORP was observed in individuals with normal sperm concentration (>15 million/mL) when compared to individuals with lower (<15 million/mL) sperm concentration. In addition, an 8-fold decrease in the sORP/cORP ratio was observed in individuals with normal sperm motility (>40%) as compared to individuals with poor sperm concentration and motility.
Conclusions: sORP in the semen samples of infertile men correlated well with their sperm concentration, motility, and morphology. A significantly high sORP/cORP ratio as assessed by MiOXSYS in infertile patients with low sperm concentration and motility suggests the role of increased oxidative stress. This newer system (MiOXSYS) measuring ORP could potentially serve as an important additional indicator in the workup of male factor infertility.

48

CAN CAFFEINE AND MELATONIN IMPROVE THE QUALITY OF FROZEN-THAWED HUMAN SPERM?

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(Presented By: Juliana Risso Pariz, BSc, MSc)

Introduction: Human semen cryopreservation has been widely used as a method of preservation of fertility. In order to reduce the damage caused by the freezing process, many studies used antioxidants and stimulants, however, its effectiveness and its role in the control of functional sperm is not well established in literature.
Objective: To evaluate the effect of melatonin (MEL) and caffeine (CAF) supplementation in structural and functional characteristics in pre-freeze and post-thaw seminal samples.
Methods: This prospective study used 10 semen samples of patients between 22 and 54 years old. All samples were normozoospermic in according to World Health Organization (WHO) criteria. The samples were cryopreserved using Human Tubal Fluid modified without supplement or with melatonin 2 mM. After thawing, the samples were analyzed or supplemented with caffeine 2 mM and incubated for 15 minutes before analysis. At the end of the experiments, we obtained four groups: post-thaw samples without supplementation, post-thaw samples supplemented with MEL, CAF and MEL+CAF. Sperm count, motility, reactive oxygen species (ROS) and mitochondrial activity were evaluated by one-way analysis of variance with repeated measures followed by post-test Holm-Sidak and the effect of cryopreservation on these parameters by paired Student's t test. Was adopted an alpha of 5%.
Results: We observed a significant reduction in total and progressive motility and decrease of mitochondrial activity in port-thaw samples ($p <0.001$). When supplemented with CAF+MEL, we observed improve in total progressive sperm count ($p<0.041$), total motile sperm count ($p<0.017$) and total motility ($p<039$). When only used CAF, a significant increase of progressive motility when compared to the post-thaw samples ($p=0.026$) was observed. Finally, total and progressive motility was higher in the group that received supplementation with melatonin than the post-freeze group ($p=0.017$). No significant difference was observed in ROS and mitochondrial activity post-thaw ($p>0.05$).
Conclusion: To date, we conclude that cryopreservation has negative effects on semen quality, reducing principally of motility parameters and mitochondrial activity and that supplementation of association with caffeine and melatonin proved to be an important tool to be applied in post-thaw samples.
Funding: Capes/DS, CNPq and Androscience.

49

MELANOMA ANTIGEN PROTEIN MAGEC1 MUTATION IDENTIFIED IN FAMILIAL NON-OBSTRUCTIVE AZOOSPERMIA

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(Presented By: Alexander W. Pastuszak, MD, PhD)

Introduction: Many currently unrecognized genetic etiologies of male infertility exist, and we sought to identify genes influencing male infertility using next-generation sequencing approaches. The melanoma antigen (MAGE) family of genes may function in germ cell maintenance and fertility, and herein we present genomic data associating the melanoma antigen gene MAGEC1 with male fertility.
Methods: Whole-exome sequencing of genomic DNA from 2 Saudi Arabian brothers with non-obstructive azoospermia (NOA) and Sertoli cell only (SCO) histology was performed. Sequence data were filtered against several genomic databases and deleterious SNPs common to both brothers identified. Sanger sequencing confirmed SNPs of interest and assessed their presence in other NOA men. Array comparative genomic hybridization (aCGH) analysis of 22 NOA men with normal karyotypes and Y-chromosome microdeletion assays assessed for gene dosage changes due to copy number variants (CNVs), and the presence of additional CNVs in genes of interest was evaluated using qPCR. Immunohistochemical staining of testis sections used commercially available antibodies and standard

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protocols.
Results: An A>T SNP at position 3530 in exon 4 of the MAGEC1 gene resulting in a potentially deleterious R1082W mutation was identified in a set of 6 SNPs mapping to 6 unique genes selected from over 300,000 SNPs shared between the two brothers' genomes. Sanger sequencing confirmed the SNP in the two brothers but did not identify it in 173 additional NOA or 34 fertile control men. The SNP was not identified in the Thousand Genomes database and is present with an allele frequency of 0.009% in the CHARGE-S database. aCGH of 22 NOA men did not identify CNVs in MAGEC1, nor did qPCR-based analysis of 84 additional NOA men. Immunohistochemical localization of MAGEC1 in human testis from a male with normal spermatogenesis revealed cytoplasmic staining of spermatocytes, which was less prominent in testis from a male with hypospermatogenesis and was absent in the testis from an SCO male.
Conclusions: A potentially damaging SNP in MAGEC1 was identified in related infertile brothers with NOA. Future work elucidating the molecular role of MAGEC1 in male fertility using cell-based assays and animal models will further our understanding of the MAGE genes in male germ cell maintenance and fertility.

50

ELECTROEJACULATION PERFORMED IN PATIENTS WITH SPINAL CORD INJURIES – A SINGLE CENTER 21 YEARS EXPERIENCE

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(Presented By: Timo Soeterik, BSc)

Introduction: During the last decades, electroejaculation (EEJ) has become an acceptable form of treatment for patients suffering from anejaculation as a result of a spinal cord injury (SCI). Although most EEJ procedures result in ejaculation, not all ejaculates contain (progressively motile) spermatozoa. When a semen sample yielded after a first EEJ does not contain progressively motile spermatozoa, a second EEJ can be considered. However, there is no consensus on whether repeated EEJ has a positive influence on semen quality.
Methods: A retrospective chart analysis was performed of all SCI patients treated with EEJ at our center from January 1994 to January 2015. Data were collected on the patients' general medical history, hormonal profiles and ultrasonographic examination of scrotum and prostate. All patients underwent EEJ according to Seager. We evaluated sperm quality according to WHO-standards, pregnancy and delivery rates. Furthermore, we investigated the effect of repeated EEJ in patients in whom the first EEJ procedure had failed.
Results: 229 EEJ procedures were performed in 47 patients. In 226/229 EEJs (98.7%) an ejaculate could be obtained. In 168/229 (73.4%) EEJ procedures it was possible to yield semen containing progressively motile spermatozoa. Median total motile sperm count (TMS) was 4.6 × 10^6 progressively motile spermatozoa [IQR 1.9 – 19.7 × 10^6 progressively motile spermatozoa]. In 18/47 (38.3%) patients, no semen of sufficient quality was yielded during the first EEJ. After repeated EEJ, progressively motile spermatozoa could be obtained in 7 of these 18 patients (38.9%). Procreation was attempted via IVF/ICSI in 15 patients (and their spouses). Mean fertilization rate per couple treated with IVF was 54.0% (SD: 17.0%) and per couple treated with ICSI 61.2% (SD: 18.2%). Pregnancy/cycle rate in the IVF and ICSI group were 1/9 (11.1%) and 11/26 (42.3%), respectively. In total, 12/15 (80%) couples achieved pregnancy. Live births of nine healthy children were reported, two pregnancies are still ongoing and one pregnant couple was lost to follow-up.
Conclusions: In the majority of patients (77%) treated with EEJ at our clinical center it was possible to obtain semen of sufficient quality which can be successfully used in artificial reproductive technologies (ART). Performing a second EEJ should be considered when no progressively motile spermatozoa are yielded during the first attempt.

51

SEMINAL OXIDATIVE STRESS, DNA DAMAGE AND TELOMERE DYNAMICS: ROLE IN IDIOPATHIC MALE INFERTILITY

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(Presented By: Swetasmita Mishra, MSc)

Introduction: Sperm DNA integrity is important for accurate transmission of genetic information to the offspring. There are different molecular factors that explain the origin and impact of sperm DNA damage. Telomeres are highly conserved hexameric repeats part of sperm DNA, which confer chromosome stability & maintain genomic integrity. Telomerase a reverse transcriptase maintain telomere length. As telomeres are Guanine rich repeats, they are highly prone to oxidative damage. Sperm transcript & DNA repair enzymes have a role in DNA integrity. The main objective of this study was to evaluate seminal oxidative stress, sperm DNA damage, sperm telomere length, telomerase activity & expression of DNA repair genes in infertile men.
Methods: The study included 165 infertile men and 102 controls. Reactive oxygen species (ROS) estimation was done by chemiluminesence method. The average telomere length from the sperm DNA was measured using a quantitative Real Time PCR. 8−hydroxy−2−deoxy−guaonosine level (8-OHdG) was assessed by Cayman's ELISA kits. DNA fragmentation index (DFI) was assessed by Sperm Chromatin Structure Assay (SCSA). Microarray of sperm transcript was done & genes selected were validated by real time PCR.
Results: The mean ROS was significantly elevated in cases compared to controls ($p<0.0001$). The 8−OHdG level in patients were significantly higher than control ($p<0.0001$). The mean %DFI in patient was higher controls. The mean telomere length was significantly lower in patient group (ROS>35) as compared to patient group (ROS<25) but it significantly increased in the

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patient group (ROS=25-35) as compared to patient group (ROS<25). 1077 genes were dysregulated from which 282 were up regulated &790 were down regulated.
Conclusion: In this study we found that in infertile patients oxidative stress leads to sperm DNA damage and telomere shortening. Elevated ROS levels lead to telomere shortening but seminal ROS to a particular level (ROS=25-35 RLU/sec/million sperm) are protective in maintaining telomere length. As an important pathway in the DNA damage repair network, genes of repair mechanisms also play a critical role in the maintenance of genome integrity.

52

GPR56-NULL MALE MICE ARE SUBFERTILE AND HAVE DEFECTIVE SPERMATOGENESIS, DECAPITATED SPERM, REDUCED MOTILITY, AND PROVIDE EVIDENCE FOR THE EXISTENCE OF A SPERM QUALITY CONTROL MECHANISM IN THE EPIDIDYMIS

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(Presented By: Laura Peyton Ellis)

Introduction: The G protein-coupled receptor 56 (GPR56) is a cell surface receptor that is widely expressed and mediates a variety of cell adhesion functions, including the migration of neural progenitor cells and the rate of melanoma metastases. In knockout mouse models, the absence of GPR56 affects testis development which results in subfertility and reduced testis size (Chen et al., 2010). However, the specific nature of this subfertility was not understood, therefore the goal of this study was to more fully characterize the Gpr56-null phenotype.
Methods: In order to confirm the effect of the Gpr56-null genotype on fertility and to further explore the difference between fertile and infertile knockouts, we performed a fertility study. All wild-type and Gpr56 heterozygous males were fertile (n=10) but only 3/10 Gpr56-null mice were fertile. Further, infertile Gpr56-null mice had significantly reduced testis size compared to fertile Gpr56-null, heterozygous, and wild-type mice.
Results: Light and electron microscopy of testes showed that infertile Gpr56 knockouts had altered testis development and interrupted spermatogenesis, as well as defects in the basement membrane and other cellular structures. Cauda epididymides of Gpr56-null mice had lower sperm densities, abundant residual bodies with excess cytoplasm from elongate spermatids, reduced sperm motility, and increased numbers of separated heads and tails. Further evaluation of head/tail separation showed that in the infertile knockout epididymides, both caput and cauda sperm had high percentages of head/tail separation, suggesting that the separation occurred prior to sperm reaching the caput. Surprisingly, in wild-type and heterozygous mice, head/tail separations were common in caput epididymides, but were significantly decreased in cauda epdidymides, suggesting that a quality control mechanism is working in wild-type mice but is defective in Gpr56-null mice. In sum, Gpr56 has an important role in multiple steps of male fertility, including testis development, spermatogenesis, sperm function, and possibly an epididymal sperm quality control mechanism.

53

PRIOR FERTILITY-SPECIFIC HEALTHCARE UTILIZATION OF COUPLES SEEKING UROLOGICAL EVALUATION FOR MALE INFERTILITY

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(Presented By: Christopher Daniel Gaffney, BA)

Introduction: We sought to determine clinical characteristics, practice patterns and prior fertility-specific healthcare utilization of couples seeking urological evaluation for male infertility.
Methods: We performed a retrospective chart review of male partners of subfertile couples who presented to a single fellowship-trained urologist at a tertiary medical center in New York between July 2012 and June 2015. Semen analysis was performed and interpreted according to 2010 WHO criteria.
Results: We identified 484 couples. The mean ages of the men and their partners were 37.8 +/- 7.6 and 34.3 +/- 5.7 years, respectively. 81.4% of couples were evaluated by a reproductive endocrinologist (RE) or OB/GYN prior to urologic consultation. 15.9% of men had already seen another urologist and were seeking a second opinion. Men referred from an RE had lower sperm concentrations and lower overall and progressive motility as compared to men who were not referred from an RE (Table). Upon presentation, 15.1% and 10.5% of couples had attempted intrauterine insemination (IUI) and in-vitro fertilization (IVF), respectively. 70.0% (51/73) of couples that had previously failed IUI and 68.6% (35/51) of couples that had failed IVF had not had a urologic evaluation prior to utilization of ART.
Conclusions: In most cases, male partners of subfertile couples seek consultation after the evaluation of the female partner. Utilization of IUI and IVF without urological evaluation of the male partner is common. Our data warrant validation in other cohorts, but suggest that there may be opportunity for earlier and more frequent collaboration between urologists and REs.

	Referred from RE		Not referred from RE		Comparison of Semen Parameters
	Mean	St. Dev.	Mean	St. Dev.	p-values
Semen Volume (ml)	3.1	3.3	2.7	2.0	p = 0.09
Semen Concentration (million/ml)	25.5	38.0	38.8	45.8	p = 0.006
Overall Motility (%)	37.9	20.9	46.3	20.4	p = 0.0006
Progressive Motility (%)	21.9	21.1	34.7	21.9	p < 0.0001
Strict morphology	3.5	7.6	3.7	6.7	p = 0.4

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54

IS THE AREA OF THE GENITAL CORTICAL REPRESENTATION ASSOCIATED WITH COPULATORY BEHAVIOR PERFORMANCE IN MALE RATS?

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(Presented By: Diana Carolina Esquivel Franco, BS)

In mammals, male copulatory behavior is far from stereotyped. In male rats, for instance, non-copulatory and copulatory phenotypes can be identified. Furthermore, male rats displaying copulatory behavior may have short, normal or long ejaculatory latencies. There are even phenotypes that feature vigorous mounts and intromissions but not ejaculation. Although all these male categories may result from interindividual differences in the availability of some neurotransmitters, receptors for sex steroids and distinctive aromatase activity in specific brain regions, it has been also shown that they might reflect differences in sensory functions such as those observed in the olfactory system between non-copulatory and copulatory rat males. However, the male sexual response depends not only on olfactory stimuli but also upon adequate somatosensory stimulation from the genitals and at the rest of the body. Thus, interindividual differences of male copulatory behavior could also result from individual anatomical and functional distinctions along somatosensory pathways. That is why here we studied the possible relationship between the size of the genital representation in the primary somatosensory cortex (S1) and the individual expression of the copulatory behavior in male rats. We assumed that males presenting copulatory patterns and short ejaculation latencies would have an over-represented genital area in S1. In track with these expectations, our preliminary results show that males having short (>600seg) and intermediate (601-1200seg) ejaculation latencies had larger genital representations in S1 than those having long ejaculation latencies (1201-1800seg), followed or not by ejaculation. In addition, non-copulatory animals had the smallest genital representations. There was an inverse correlation between the area of the genital cortical representation and ejaculation latencies among the groups of male rats studied, thus suggesting that the better the performance, the larger the S1 genital representation.

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56

EVALUATION OF ELECTROMAGNETIC FIELD EFFECT ON OXIDATIVE STRESS LEVEL, TESTOSTERONE HORMONE AND SEXUAL BEHAVIOR IN MALE RAT TREATED WITH OMEGA 3

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(Presented By: Nava Ainehchi, PhD)

Introduction: Omega-3s are considered essential fatty Acids that cannot be synthesized by human body and should be obtained from foodstuff. This study aimed at evaluating the beneficial effect of Omega-3s, exposed in Electromagnetic Field (EMF), on sexual behavior ,Serum total testosterone level, malondialdehyde (MDA) level and total antioxidant capacity(TAC) in male rats.
Methods: 32 Wistar rats (males=24, females=8) were allocated to four groups of Control, EMF, omega 3 and omega 3 & EMF. Estradiol benzoate was injected to the female rats subcutaneously. Then after taking biopsy from epididymis and testis of each groups, tissue preparation was performed to look through via light Microscope on 28th day of study. Serum MDA, TAC and Testosterone were measured in male rat by Radio Immune Assay (RIA) method.
Results: The highest degree of testicular tissue destruction and MDA level were observed in electromagnetic field group and the lowest in omega 3 group. The sexual behavior, testosterone hormone and TAC level were lowest in electromagnetic field group and highest in omega 3 group.
Conclusion: Based on the results, administration of omega 3 can significantly lower the adverse effects of EMF and have beneficial influences on sexual behavior in male rat.

57

UNDERLYING SEXUAL DYSFUNCTION IN MEN PRESENTING WITH VARICOCELE

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(Presented By: Matthew J. Pagano, MD)

Introduction: Varicoceles are found in about 15% of adult men and generally follow an indolent course. Most are incidentally discovered or found during infertility workup, and only a minority cause pain (2-10%). There are few reports on the effect of varicocele on overall sexual health. We sought to investigate the association of varicocele with self-reported domains of sexual function.
Methods: All cases presenting to a single andrologist with chief complaint of varicocele from 1/2013 to 7/2014 were retrospectively reviewed. Prior to assessment or treatment all men completed the validated, self-administered Male Sexual Health Questionnaire (MSHQ), a 25-question survey on erections, ejaculation, satisfaction and sexual activity. Results were compared to healthy men presenting during the same period for vasectomy consultation. Differences in sexual domain scores were assessed by Mann-Whitney U test.

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Results: Men with varicocele (n=44) were younger (median age 32 vs 38, p<0.001) and less likely to be in stable monogamous relationships (19/44 vs 21/21, p<0.001) compared with controls (n=21). Overall sexual satisfaction domain scores (scale 6-30) were lower in men with varicocele (median 25 vs 29, p=0.039). Patients with varicocele were less satisfied with the overall sexual relationship with their main partner (median 4 vs. 5, p=0.019), quality of sex life with their main partner (median 4 vs.5, p=0.007), and had a lower desire to have sex with main partners (median 3 vs. 4, p=0.017) compared to control patients. Erection scores were similar and normal with a median of 15 points in both groups (scale 0-15). Total ejaculation scores were also similar in both groups with a median of 32 in the varicocele group and 33 in controls (scale 1-35). Varicocele patients reported statistically more dysorgasmia when compared to controls. Nine out of 44 (20%) reported "slight" or "moderate" pain with ejaculation, as compared with zero controls (p=0.022). This did not seem to be related to baseline scrotal pain from varicocele as only 1/3 of patients with dysorgasmia reported baseline scrotal pain.
Conclusions: Despite no measurable effect on overall erectile or ejaculatory function, patients with varicocele reported lower sexual satisfaction, lower sexual desire, and dysorgasmia. This impact on sexual quality of life suggests that evaluation of factors outside of typical functional domains may be needed to fully address the concerns of these patients.

58

OUTCOMES OF INFLATABLE PENILE PROSTHESIS SURGERY: AN ANALYSIS OF A NEW YORK STATE DATABASE

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(Presented By: Ifeanyi Cliff Onyeji, BA)

Introduction: Population-level data on the outcomes of inflatable penile prosthesis (IPP) surgery is limited. We sought to examine patient and surgeon-level factors associated with infectious and mechanical failure after IPP surgery in New York State and to delineate statewide patterns in the management of these complications.
Methods: The New York Statewide Planning and Research Cooperative System (SPARCS) database was queried for IPP cases from 1995–2014. Initial cases, subsequent reoperations and their indications were identified with CPT and ICD-9 CM procedure and diagnosis codes. Reoperations were tracked for up to five years. A proportional hazards regression was used to identify risk factors for infectious and mechanical complications. Assessed factors included clinical characteristics and demographic data and annual surgeon volume (threshold ≥ 20 implants).
Results: The cohort included 13,684 cases of first time IPP insertion. The rates of reoperation due to infectious and mechanical complications were 1.94% and 2.94%, respectively. Medicaid insurance (vs. private) and low annual surgeon volume significantly predicted both infectious (HR 1.732, 95% CI 1.045 - 2.870; HR 2.353, 95% CI 1.722 - 3.215) and mechanical failure (HR 2.030, 95% CI 1.353 - 3.048; HR 1.303, 95% CI 1.037 - 1.637). Diabetes predicted infectious reoperation (HR 1.488, 95% CI 1.141 - 1.939). Most infectious complications were managed with explantation where as IPP removal with immediate IPP replacement was the dominant treatment for device malfunction.
Conclusion: Low-volume surgeons, Medicaid recipients and diabetics had higher rates of infectious complications after IPP. Only the former two were associated with mechanical failure. The majority of malfunctioned IPPs were replaced with new IPPs, as opposed to semi-rigid implants, while most infected IPPs were explanted without replacement.

Table 1. Multivariable analysis of risk factors for infectious and mechanical reoperation

	Reoperation for IC		Reoperation for MC	
Predictor	HR (95% CI)	p-value	HR (95% CI)	p-value
Diabetes	1.488 (1.141 - 1.939)	0.003	-	-
Insurance				
Private	REF	1	REF	1
Medicare	1.538 (1.141 - 2.073)	0.005	-	-
Medicaid	1.732 (1.045 - 2.870)	0.033	2.030 (1.353 - 3.048)	0.001
Annual surgeon volume				
High (≥ 20)	REF	1	REF	1
Low (< 20)	2.353 (1.722 - 3.215)	<0.001	1.303 (1.037 - 1.637)	0.023

*IC = infectious complication; MC = mechanical complication

Figure 1. Management of infectious and mechanical complications.

59

FOUR-RING CIRCUS: A CASE OF PENILE INCARCERATION

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(Presented By: Graham Luke Machen, MD)

Introduction: Penile incarceration, although rare, presents as a true urologic emergency. The first case of a penile constriction device resulting in incarceration was reported in 1755. Most commonly, these devices are placed for enhancing and/or prolonging erections, as they obstruct venous return in the penis. If left untreated, this may result in ischemia of erectile tissues. Recently one such patient was encountered in our ED. We describe our experience in removing the constriction devices at the bedside.
Methods: The patient was a 63-year-old male, who, after placing four 2" stainless steel washers around the base of his penis, proceeded to fall asleep. The next day he was unable to remove the objects, and sought treatment in the ED. The patient admitted that he placed the objects to increase the caliber of his erection. On examination, his penis had swollen dramatically. Measures to remove the washers included the string method using a 1/2" Penrose drain and an 0-silk tie, bolt cutters, and a battery-powered bone saw. All were unsuccessful. A hack saw was procured, but only partially incised the washers. Ultimately, the Fire Department was called, and personnel came to the ED with a Dremel saw. The patient was draped with wet towels and cystoscopy tubing was set up to continuously soak the saw to prevent burning the patient. Pliers were placed between the

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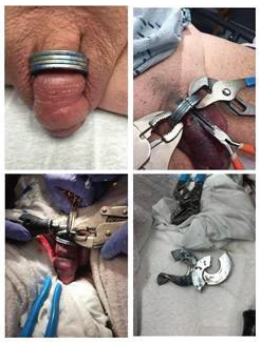
washers and the penis. A wedge of each washer was resected, and all the washers were removed.

Results: As previously mentioned, all four washers were safely removed at bedside. The penis sustained no trauma aside from some abrasions secondary to the edema. The patient was discharged from the ED after voiding spontaneously.

Conclusion: There are a myriad of treatment modalities to choose from to remove metallic devices encircling the penis, but no consensus exists as to which may be best. In this report, the patient had a grade I penile incarceration based on the scale proposed by Bhat et al. Several of the treatment options were attempted before having success with the Dremel saw. Regardless of the grade of injury or intervention required, early diagnosis and treatment of this condition are essential in avoiding potential complications.



60

CLAUDINS ARE ALTERED DURING GONOCYTE TO SPERMATOGONIA TRANSITION AND IN SEMINOMAS

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(Presented By: Gurpreet Manku, BSc, PhD)

Introduction: Testicular germ cell tumors (TGCTs) are the most common type of cancer in young men and their incidence has been steadily increasing for the past decades. TGCTs and their precursor carcinoma-in-situ (CIS) are thought to arise from the deficient differentiation of gonocytes, precursors of spermatogonial stem cells. Gonocytes are present early during spermatogenesis and undergo various well-timed and strictly regulated processes including proliferation, migration to the seminiferous tubule basement membrane, differentiation and apoptosis. Although we have previously shown that gonocyte differentiation is induced by retinoic acid (RA) and that Gadd45a and Cycs are likely involved in apoptosis of improperly developing gonocytes, the mechanisms relating failed gonocyte differentiation to CIS formation are unknown. The goal of the present study was to uncover genes regulated during gonocyte development that would show abnormal patterns of expression in testicular tumors, as prospective links between failed gonocyte development and TGCT formation.

Methods: To identify common gene and protein signatures between gonocytes and seminomas, we first performed gene expression analyses of transitional rat gonocytes, spermatogonia, human normal testicular and TGCT specimen.

Results: Gene expression arrays, pathway analysis and quantitative-real time PCR analysis identified cell adhesion molecules as a functional gene category including genes downregulated during gonocyte differentiation and highly expressed in seminomas. In particular, the mRNA and protein expressions of claudins 6 and 7 were found to decrease during the gonocyte transition to spermatogonia, and to be abnormally elevated in seminomas. The dynamic changes of these genes suggest that they may play important physiological roles during gonocyte development. Moreover, our findings support the idea that TGCTs arise from a disruption of gonocyte differentiation, and position claudins as interesting genes to further study in relation to testicular cancer.

61

HUMAN SPERM NUCLEAR MORPHOMETRY FOR SEMEN QUALITY ASSESSMENT AS MEASURED WITH FOURIER HARMONIC ANALYSIS

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(Presented By: Megan Krautkramer, MS)

Introduction: Sperm chromatin integrity and accurate packaging are essential to successful fertilization and are therefore useful assessments of male fertility. Sperm nuclear shape reflects these qualities and has been measured with Fourier harmonic analysis (FHA) which describes shape in terms of harmonic amplitudes (HA; in microns).

Methods: Semen samples (n=191) were stained with Hoechst 33342, applied to slides via a Statspin Cytofuge® and imaged under fluorescent microscopy. Images were analyzed with ImageJ macros adapted for edge detection and measurement for human sperm. Nuclear shape was characterized with HAs and assessed for conventional morphology via the Kruger method according to the World Health Organization (WHO, 2010). An average nuclear shape was derived to serve as a template representing the average sperm nuclei (mean ± standard error of the mean; HA0 = 1.856 ± 0.006; HA1 = 0.045 ± 0.001; HA2 = 0.383 ± 0.007; HA3 = 0.0589 ± 0.001; HA4 = 0.079 ± 0.002; HA5 = 0.031 ± 0.001; perimeter = 13.4 ± 0.1; length 4.8 ± 0.03; width 3.2 ± 0.01). Cluster analysis grouped ejaculates based on HA0-5, resulting in eight clusters. The data from the average nuclear shape of all ejaculates was manipulated by replacing HA0-5 with the mean HA0-5 of each cluster and Cartesian coordinate graphs were mapped representing the average shape per cluster. Bonferroni's pair-wise t-test between HAs of each cluster allowed numerical comparison. The number of

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comparisons differing between clusters per HA allowed conclusions to be drawn about the components of shape that defined each cluster and which HAs contributed most to variation within the population of ejaculates.
Results: Pearson's correlation revealed that HA1-5 negatively correlated with percent normal morphology ($P < 0.0001$) but HA0 did not ($P > 0.05$). Greater HAs corresponded to lower percent normal morphology (9.4% ± 0.46) and more pinching in the posterior region of sperm. Mean HA0 varied more between the eight groups than all other HAs (24 of 28 comparisons differed; $P < 0.05$). The power of the FHA approach is that it produces sensitive, objective and orthogonal measures of sperm nuclear shape. The resulting FHA parameters provide a potential means to compare sperm shape not only to fertility, but also nuclear difference that may be associated with factors such as age, environmental contaminants, disease state, diet or heat stress. Future studies should include fertility data, to investigate an association to clusters and each HA.

62

COMET DISTRIBUTION PLOT: PROVIDES UNIQUE PATIENT PROFILING ALONG WITH HIGH DIAGNOSTIC VALUE

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(Presented By: Luke Simon, PhD)

Introduction: The output parameters of the alkaline Comet assay mean DNA damage of all comet tail (meanDD), olive tail movement (OTM) and the percentage of sperm with damage (%damage) have been used frequently to determine male reproductive health and to predict assisted reproductive technology (ART) success.
Methods: In this study, using 47 fertile donors and 238 patients undergoing infertility diagnosis, including 132 couples undergoing ARTs, each Comet profile was analyzed to develop "Comet distribution plot" and categorize them into three categories.
Results: All fertile donors exhibited type A distribution, while type A, B and C distribution was observed in 44.96%, 40.76% and 14.29% of the patients, respectively. Type B distribution was further categorized into two types, B1 when the peak is between 26-50% damage (37.11%) and B2 when the peak is between 51-75% damage (62.89%). Fertile-donors had low meanDD (14.56±0.77 versus 40.01±1.49; $P<0.001$), low OTM (2.01±0.17 versus 5.51±0.36; $P<0.001$), low %damage (20.77±1.56 versus 61.50±1.86; $P<0.001$) and type A plot (100% versus 44.5%; $P<0.001$) compared with infertile patients. Comet parameters were associated with clinical pregnancies where successful couples had low meanDD (37.81±2.59 versus 49.04±3.64; $P=0.012$), low OTM (6.92±0.75 versus 10.10±0.99; $P=0.011$), low %damage (35.29±3.25 versus 24.67±4.09; $P=0.043$) and plot A and B1 distribution (82% versus 63%; $P=0.05$) when compared to unsuccessful couples. About 66% of couples with type A distribution plot were successful after ARTs, whereas couples with type B1, B2 and C distribution plots resulted in 56%, 44% and 33% pregnancies, respectively. A combinational analysis of all the Comet output parameters may provide a comprehensive evaluation of patient's reproductive health as these parameters measure different aspects of DNA damage within the sperm.

63

WITHDRAWN

64

EXPRESSION OF A 52 KDA PEPTIDE FROM MUCIN-5B IN THE SEMINAL PLASMA AS A MEASURE FOR MONITORING SEMINAL OXIDATIVE STRESS

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(Presented By: Paula Intasqui Lopes, MSc, BSc)

Introduction: Seminal oxidative stress has been considered a major mechanism underlying male infertility. However, methods currently used for its evaluation still present poor correlation with male fertility status. Taking this into consideration, a previous proteomic study from our group identified the Mucin-5B (MUC5B) protein as a potential seminal biomarker of semen lipid peroxidation (LPO), a measure of oxidative stress. Mucins are gel-forming proteins and several studies have demonstrated that these proteins are targets for kallikreins (KLK) proteolytic activity. Therefore, we hypothesized that MUC5B, when cleaved by KLK3 in the seminal plasma, forming a 52 kDa peptide, may be useful for monitoring semen oxidative status. Thus, our aim was to investigate if this peptide expression may reflect seminal oxidative stress in different conditions.
Methods: A cross-sectional study was performed in four groups: low LPO (LPO levels <17.39 nmol/mL, n=22), high LPO (LPO levels >29.34 nmol/mL, n=20), control (n=16, normozoospermic men without infertility factors) and varicocele (n=15, normozoospermic men presenting only varicocele). The first two groups were composed by normozoospermic men selected in our previous study. Semen was analyzed according to WHO 2010 guidelines and centrifuged for seminal plasma separation. LPO levels were assessed using a colorimetric assay for malondialdehyde. MUC5B was quantified individually in each group using western blot (52 kDa peptide quantification, data normalized by a loading control) and dot blot (quantification of total MUC5B protein and cleaved peptides) analyses. Groups were compared by one-way ANOVA (Sidak post hoc) or Kruskal-Wallis (Games Howell post hoc).
Results: Results are presented in Table 1. LPO levels differed between all groups. Total MUC5B expression was not different between the groups. However, an overexpression of the 52 kDa peptide was observed in the low LPO group.
Conclusion: MUC5B 52 kDa peptide is overexpressed only in samples presenting low LPO, and it is not associated with other conditions, such as varicocele, suggesting that this peptide may be a good marker for monitoring semen oxidative state.
Funding: FAPESP (2014/11493-0, 2014/17185-6).

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Table 1. Semen analysis and expression of MUC5B and a 52 kDa peptide from MUC5B in the seminal plasma. Data are expressed as Mean; Standard Deviation, and groups were compared using one-way ANOVA followed by a Sidak *post hoc* test, unless otherwise described.

	Low lipid peroxidation (n=22)	High lipid peroxidation (n=20)	Control (n=16)	Varicocele (n=15)	p
Age (years)	35.2; 5.52	36.9; 6.74	32.3; 4.99	32.9; 4.17	0.059
Volume (mL)	3.8; 1.54	3.5; 1.70	3.4; 0.92	3.3; 1.38	0.760
Sperm concentration ($x10^6$/mL)	49.9; 74.55	81.4; 69.58	57.8; 106.65	50.0; 35.50	0.532 [£]
Sperm progressive motility (%)	54.0; 7.58	48.4; 7.62	53.1; 7.35	54.0; 8.06	0.071
Sperm morphology (% normal)	7.4; 2.98	7.2; 2.07	6.7; 2.33	5.9; 1.13	0.236
Round cells ($x10^6$/mL)	1.3; 0.98	1.7; 1.46	1.0; 0.71	1.1; 1.05	0.270
Neutrophils ($x10^6$/mL)	0; 0.21	0; 0.13	0; 0.10	0; 0.08	0.213 [£]
Lipid peroxidation (nmol/mL)	14.9; 1.60 [a]	32.0; 2.82 [b]	24.9; 3.97 [c]	21.2; 5.62 [d]	**<0.001** *
52 kDa peptide from MUC5B (western blot, a.u.)	1.7; 0.89 [a]	1.1; 0.59 [b]	0.9; 0.33 [b]	1.0; 0.35 [b]	**<0.001** *
MUC5B (Log dot blot, a.u.)	6.3; 0.69	5.9; 0.71	6.0; 0.72	5.8; 0.62	0.314

[£] - Groups compared by a nonparametric Kruskal-Wallis test, followed by Games-Howell *post hoc* test. Values expressed in median; interquartile range.
* - Statistically different between the groups ($p<0.05$).
Different superscript letters in a same row indicate a statistical difference (*post hoc* test <0.05).
a.u. – arbitrary units.
Western blot values were normalized by a loading control.

65

MULTIPLE MECHANISMS OF SPERM ASSOCIATED ANTIGEN 6 (SPAG6)-ASSOCIATED MALE FERTILITY: MOTILITY AND SPERMATOGENESIS

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(Presented By: Hong Liu, Master)

Mammalian SPAG6 is the orthologue of PF16, a component of the central apparatus of the "9+2" axoneme of Chlamydomonas reinhardtii. Mutations in the Chlamydomonas PF16 gene cause flagellar paralysis. It has been reported that approximately 50% of Spag6-deficient mice died from hydrocephalus before adulthood, and males surviving to maturity were infertile due to impaired sperm motility in the original C57/SV129 genetic background. Our recent studies demonstrated that SPAG6 also regulates cell proliferation, migration, division, polarity and ciliogenesis. Given that surviving Spag6-deficient mice had significantly reduced sperm number and abnormal sperm axoneme ultrastructure, we hypothesized that SPAG6 also regulates spermatogenesis. Testis histology was examined in the Spag6-deficient mice during the first wave of spermatogenesis. A significant developmental delay was observed from day 16 after birth. SPAG6 was present as vesicles in spermatocytes and in the acrosome region and the manchette of spermatids. Mouse SPAG6 was used as bait for a yeast two-hybrid screen, Snapin was identified to be the most frequent partner. Snapin is also one of the strongest binding partners of intraflagellar transport 20 (IFT20), a key spermiogenesis regulator. Snapin is ubiquitously expressed in vivo. However, the expression level is the highest in the testis among the tissues analyzed. During the first wave of spermatogenesis, Snapin expression is dramatically increased at day 30, suggesting a role in spermiogenesis. In male germ cells, Snapin is also present as vesicles in spermatocytes, and highly concentrated in the acrosome of round spermatids and manchette in elongating spermatids. These localizations are almost identical to SPAG6 and IFT20, suggesting that SPAG6, Snapin and IFT20 form a complex. Besides Snapin, several other proteins, including Spink2 gene, which belongs to the SPINK family of proteins characterized by the presence of a Kazal-type serine protease inhibitor-pancreatic secretory trypsin inhibitor domain also appeared multiple times. A mouse model carrying a Spink2 gene trap mutation exhibited disrupted testicular integrity, elevated serine protease activity, reduced sperm number, and impaired fertility. Overall, our studies suggest that SPAG6 not only regulates sperm motility, it also controls spermatogenesis by multiple mechanisms through binding to multiple proteins via its eight contiguous armadillo repeats that mediate protein-protein interaction.

66

IDENTIFICATION OF THE SOURCE OF CHOLESTEROL USED FOR STEROID BIOSYNTHESIS IN PRIMARY RAT LEYDIG CELLS

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(Presented By: Sathvika Venugopal)

Introduction: The hormone-sensitive and rate-limiting step in steroid biosynthesis is the movement of cholesterol from intracellular sources to the inner mitochondrial membrane (IMM) where cholesterol is metabolized to pregnenolone. Despite the numerous studies carried out to analyze cholesterol trafficking in steroidogenesis, the exact source of cholesterol as well as the mechanism by which it is transported from intracellular sources to IMM remains to be elucidated. Our studies using D4, the fourth domain of perfringolysin O protein, which has the ability to bind with high affinity to cholesterol-enriched membranes, suggested that in MA-10 mouse tumor Leydig cells the bulk of the steroidogenic pool of cholesterol, mobilized by dibutyryl-cAMP (dbcAMP) for acute steroidogenesis originates from the plasma membrane. D4 was further employed in this study to understand the source organelle for cholesterol in isolated primary rat Leydig cells during acute steroidogenesis.
Methods: D4 cDNA was inserted into the Lenti-X expression pLVX-mCherry-C1 vector (Clontech). The plasmid was then transfected into Lenti-X 293T Cell Line (Clontech) using Xfect reagent (Clontech) in the presence of Lenti-X HTX Packaging mix (Clontech). A high titer of about 108 infectious units (ifu)/ml of lenti-viruses were obtained after concentrating the media containing using lenti-X concentrator (Clontech). Testes from 60-64 days old Sprague-Dawley rats were decapsulated, enzyme digested and subjected to Percoll gradient centrifugation to obtain purified Leydig cells (90%). The cells were plated on cyclic olefin copolymer (COC) 96-well plate (Corning) and infected with concentrated lentiviral particles containing mCherry-D4. 24 hours post infection, Leydig cells were visualized under scanning confocal microscope.
Results: Over 95% of the primary rat Leydig cells were found to express the mCherry-D4. In a fraction of these cells fluorescence localized at the plasma membrane. Upon dbcAMP stimulation a significant reduction in plasma membrane labeling was observed within 60 minutes.

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Conclusion: These data suggest that plasma membrane is one of the sources of cholesterol used for steroid hormone formation in primary rat Leydig cells.
Funding: Supported by CIHR grant MOP-102647 and a CRC

67

LOW DIETARY IRON INTAKE DECREASES EPIDIDYMAL SPERM COUNTS IN MICE

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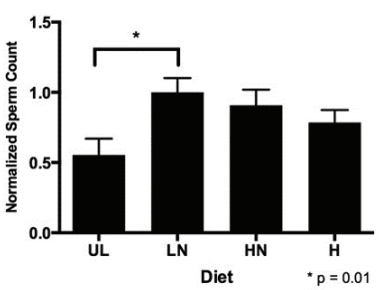
(Presented By: Joseph S. Gabrielsen, MD, PhD)

Introduction: Iron is critical for normal cellular function, yet iron excess is associated with increased oxidative stress and cellular toxicity. Spermatogenesis is an iron-dependent process, with iron-containing enzymes regulating DNA synthesis, chromatin remodeling, biogenesis of mitochondria and cellular respiration. Sertoli cells have been shown to express the full complement of iron regulatory machinery and we have recently presented evidence that iron concentrations are actively regulated in the testes of mice. These data suggest a role for maintaining optimal intratesticular iron concentrations for proper testicular function. We, therefore, sought to determine whether dietary iron intake affects spermatogenesis in mice.
Methods: Male C57Bl6 mice were fed diets containing ultra low (UL, 4 mg/kg, n=9), low normal (LN, 35 mg/kg, n=10), high normal (HN, 500 mg/kg, n=10) or high (H, 2000 mg/kg, n=9) iron content for two months following weaning. Sperm were extracted by incubating minced caudal epididymides in extraction medium. Released sperm count was determined by manual assessment using a hemocytometer. For each cohort of mice, sperm counts were normalized to the low normal group.
Results: Between 2.8 and 6.4 million sperm were obtained per mouse. There was no significant difference in sperm counts between the low-normal and high-normal diets (p=0.55). Sperm counts were 45% lower in the ultra-low compared to the low-normal diets (p=0.01). Sperm counts were 21% lower in the high compared to low-normal diet, but did not reach significance (p=0.14).
Conclusions: Low dietary iron intake was associated with a significant decrease in sperm counts in mice. High iron diet may also be associated with impaired spermatogenesis. These preliminary data suggest that regulation of intratesticular iron levels may play an important role in maintaining optimal spermatogenesis.



68

REGULATION OF DISTAL GATA4 1B PROMOTER ACTIVITY

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(Presented By: Vanessa Theberge)

The GATA4 transcription factor plays a key role in the development and function of multiple tissues derived from both mesoderm and endoderm, including the gonads. In the testis, GATA4 is highly expressed in the somatic cell lineage where it appears to regulate expression of numerous genes involved in gonadal morphogenesis (Sry, Sox9, Amh) and steroidogenesis (Star, Cyp11a1, HSD3B2). Despite these crucial roles, our understanding of the mechanisms that regulate GATA4 expression remain limited. We have shown that the GATA4 gene is expressed as multiple transcripts that differ in their first untranslated exon sequence. Two of them are predominantly expressed and are conserved among species: transcripts GATA4 E1a (generated by a proximal 1a promoter) and GATA4 E1b (via a distal 1b promoter). Since both GATA4 transcripts produce the same GATA4 protein, our hypothesis is that differential promoter usage is an important mechanism for regulating spatiotemporal GATA4 expression in the gonads and in other tissues. Our previous work using transgenic mice demonstrated that a 5 kb fragment of 1a promoter recapitulates endogenous Gata4 expression only in Sertoli cells in the male gonad. We therefore hypothesized that 1b promoter, like 1a promoter, contributes to cell- and tissue-specific Gata4 expression in other cell types in the testis. We generated transgenic mice expressing a GFP reporter under the control of a 2 kb fragment of the distal promoter. We analyzed GFP protein expression in several tissues and at many developmental stages by epifluorescence, immunohistochemistry and Western blot. Our results showed that 2 kb of Gata4 1b promoter is not sufficient to direct transgene expression in the mouse testis. Other elements required for endogenous transcription of the gene from its distal 1b promoter are required. To identify these elements, we performed an in-silico analysis of enhancer-promoter interaction using Fantom5 database and IM-PET algorithm. Four enhancers potentially involved in Gata4 1b promoter regulation were identified and cloned in a vector expressing luciferase reporter gene. Transient transfection studies are currently being used to evaluate the impact of these enhancers on GATA4 promoter activity in different gonadal cell lines. These results will lead to a better understanding of the transcriptional regulation of the GATA4 gene in different cell types and at distinct developmental

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stages.

69

MULTIPLE COMPONENTS OF THE MEIOSIS-ACTIVATING STEROL SIGNALLING AND TRANSCRIPTION FACTOR NETWORKS DIFFERENTIALLY REGULATE SERTOLI CELL GENE EXPRESSION

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(Presented By: Keumsil Hwang, BS, MS)

Introduction: Enzymatic formation of lanosterol (LAN) from squalene leads to the core structure of steroids. Gonadal LAN is a precursor of meiosis-activating sterols (MAS), factors with a role in regulating meiosis during spermatogenesis (T-MAS) and oogenesis (FF-MAS).
Objective: To evaluate transcriptional pathways involved in testicular sterol effects on Sertoli cell (SC) function, a rat SC ex vivo model was used.
Methods: 18-day-old SD rat testes were used to isolate and purify SCs. Primary cultures (≥95% pure) were maintained at 1x10e-7 cells/100mm dish. On day 3, SCs were treated short-term or 24h with 0.1-10 microM of a specific sterol or vehicle-matched controls (CT). Nuclear (Nu) and cytoplasmic (Cy) proteins and total RNA were collected from each replicate. Mitogen-activated kinase (MAPK), protein kinase B (PI3K/Akt), c-Jun N-terminal kinase (JNK), JAK-STAT and CREB (cAMP response element-binding protein) pathways were evaluated by Western Analysis with activation assessed by phosphorylation (p) status. Triplicates were analyzed for each experiment. Throughout, vehicle- and time-matched CT were present. Normalization of each phosphorylated protein was expressed relative to its respective total protein level. Q-RT-PCR was used to measure mRNAs normalized by 18S. Statistical significance, $p \leq 0.05$.
Results: At 24h, effects of MAS were compared to sterols LAN, 22-hydroxycholesterol and cholesterol. MAS alone consistently decreased steady-state activity of pJAK2, Cy and Nuc pAkt, pJNK, pSTAT3 and cellular levels of AR. At 100nM (24h), mRNA changes were receptor and sterol-specific, all sterols except cholesterol decreased AR, PR and ERbeta protein levels. LAN rapidly increased Cy and Nu activated MAPK (5min). Maximum levels of Nu pMAPK/MAPK were seen at 15min; effects persisted for 24h. CREB was rapidly phosphorylated and significant time-dependent increases in Nu pCREB/CREB seen. Interestingly, a 2-fold increase in Nu pCREB level was observed at 24h. Rapid corresponding decreases in Cy pCREB/CREB were seen. Significant, prolonged increases in Cy and Nu pAkt/Akt levels were observed (15min-6h). At 24h, Nu pAkt levels decreased in LAN-treated SC.
Conclusion: Together our findings suggest that intratesticular sterols, including T-MAS, may differentially and temporally integrate nuclear activities of specific SC transcription factors, including AR, and thereby regulate spermatogenesis.
Funding: Studies funded by NICHD and the Population Council (PLM).

70

NA/K-ATPASE REGULATES EXPRESSION OF CONNEXIN 43 AND CLAUDIN 11 IN RAT PREPUBERTAL SERTOLI CELLS

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(Presented By: Gayathri Rajamanickam, BVSc)

Introduction: Novel roles for Na/K-ATPase-ouabain (specific inhibitor) interaction have been established in Sertoli cells and in other epithelial cell types. The objective of the study was to investigate the role of Na/K-ATPase isoforms in the formation and function of junctional complexes in Sertoli cells.
Methods: Primary cultures of Sertoli cells were obtained by enzymatic digestion of prepubertal rat testis and were grown on Matrigel coated dishes for 7 days.
Results: Sertoli cells predominantly expressed the ubiquitous isoform (ATP1A1) of Na/K-ATPase which was confirmed by immunoblotting, PCR, immunofluorescence and mass spectrometry. Treatment of Sertoli cells with ouabain (50 nM) increased trans-epithelial resistance (TER), claudin 11 and connexin 43 expression while 1 mM ouabain had the opposite effects (decrease in TER, claudin 11 and connexin 43 expression). We also evaluated the involvement of Src-EGFR-ERK-CREB pathway in ouabain-mediated expression of claudin 11 and connexin 43. While incubation of Sertoli cells with 50nM ouabain increased content of p-Src, p-EGFR, p-ERK1/2, and p-CREB, 1 mM ouabain decreased phosphorylation of the above mentioned signaling molecules. Preincubation of Sertoli cells with PP2 (Src inhibitor; 10 μM) and PD98059 (MAPK inhibitor; 25 μM) inhibited ouabain induced effects on these signaling molecules, TER, and expression of claudin 11 and connexin 43.
Conclusion: Overall, the results suggest that Na/K-ATPase regulates formation and function of Sertoli cell tight junctions through Src-EGFR-ERK1/2-CREB pathway. It is noteworthy that ouabain is an endogenous steroid hormone. Therefore, its interaction with Na/K-ATPase may serve as a critical signaling mechanism involved in regulation of Sertoli cell function and male fertility.
Funding: This study received funding from Natural Sciences and Engineering Research Council (NSERC) of Canada.

71

TRANSCRIPTION FACTORS SF-1 AND CJUN COOPERATE TO ACTIVATE THE FDX1 PROMOTER IN MA-10 LEYDIG CELLS

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The Ferredoxin 1 (Fdx1) protein supports steroid biosynthesis in steroidogenic cells through electron transfer to the rate-limiting

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steroidogenic enzyme, Cyp11a1. The latter catalyzes the conversion of cholesterol to pregnenolone through side chain cleavage inside the mitochondria. Thus far, only several transcription factors have been implicated in the regulation of Fdx1 promoter activity in Leydig cells. These include the nuclear receptor SF-1 and Sp1. Since conserved regulatory elements for the AP-1 transcription factors have been located at -764 and -617 bp of the Fdx1 promoter and members of this family are ubiquitously expressed, we hypothesized that cJUN may cooperate with other partners to regulate Fdx1 in Leydig cells. Although independent of Sox transcription factors regulation, we report that SF-1 and c-JUN cooperate to activate the Fdx1 promoter in MA-10 Leydig cells using luciferase and quantitative real-time PCR assays. Furthermore, we found that such activation requires different regulatory elements located between -124 and -306 bp of the Fdx1 promoter. Thus, our data provides new insights in the molecular mechanisms that control mouse Fdx1 transcription, possibly leading to regulation of Cyp11a1 enzyme activation, in Leydig cells.

72

TRANSDIFFERENTIATION OF RAT STEM LEYDIG CELLS INTO PROSTATIC AND UTERINE EPITHELIUM, BUT NOT EPIDERMIS

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(Presented By: Manjunatha Ketumaran Nanjappa, BVSc & AH, MVSc, PhD)

Introduction: Leydig cells are testosterone secreting cells in the testicular interstitium. Stem Leydig cells (SLCs) isolated from adult rat testis can regenerate new populations of Leydig cells. Recent literature indicated that SLCs and other stem cells can give rise to new tissue types under certain conditions, but limited information exists on SLC plasticity. This study sought to determine whether rat SLCs could transdifferentiate into cell types of all three germ layers, prostatic epithelium (endoderm), uterine epithelium (mesoderm) and epidermis (ectoderm), using a tissue recombination and grafting technique.
Methods: SLCs, isolated from Leydig cell-depleted testes of adult rats, were purified and grown in vitro. SLCs were combined with fetal or neonatal mesenchyme from 16-day fetal dermis or urogenital sinus (prostate precursor) or two- to four-day postnatal uterus. Tissue recombinants were grafted under the renal capsule of nude mouse hosts and grown five weeks. To identify the source of cells in tissue recombinants, mesenchyme from green fluorescent protein (GFP)-expressing mice was used.
Results: After five weeks, tissue recombinants of urogenital sinus mesenchyme (a potent prostate inducer) and SLCs grafted in male nude mice formed fluid-filled ductal structures with a simple cuboidal epithelium typical of prostate. Epithelium was SLC-derived, as determined by absence of GFP, and expressed the prostatic epithelial marker Nkx3.1. Similarly, uterine mesenchyme/SLC recombinants grown in female nude mouse hosts contained a GFP+ stroma and columnar luminal epithelium that was GFP-negative, suggesting that it was from SLCs, and expressed estrogen receptor 1 and cytokeratin 8. In contrast, tissue recombinants of mouse dermal mesenchyme recombined with SLCs did not form epidermis, and recovered grafts consisted only of dermis. These results suggest adult rat SLCs can undergo transdifferentiation and form histologically normal prostatic or uterine epithelium expressing molecular markers for that tissue. Adult SLCs can give rise to epithelium from either male or female reproductive organs under the inductive influence of fetal/neonatal mesenchymes from other reproductive organs. Despite the ability of the mesodermally-derived SLCs to form prostatic or uterine epithelium (endodermal and mesodermal derivatives, respectively), SLCs did not form epidermis (ectoderm), suggesting SLCs may not be able to transdifferentiate into tissues derived from all germ layers.

73

TRANSLATIONAL DYNAMICS OF GERM CELL DEVELOPMENT

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(Presented By: Ana Cristina Lima, MSc)

Introduction: Several lines of evidence suggest that translational control is an important regulatory mechanism during mammalian spermatogenesis - whereby transcripts are produced and then translated days apart. The purpose, the extent and timing of this translational control and the factors involved, are relatively unknown. Deep sequencing of ribosome-protected mRNA fragments (RPFs), or ribosome profiling, provides a new way of studying translational dynamics, but the application of this method to the study of spermatogenesis has been hindered by the requirement of high quantity of starting material.
Objective: Development of a new approach for parallel deep-sequencing of both transcripts and mRNA undergoing translation from samples with low RNA input, such as germ cells in different developmental stages.
Methods: RNA was recovered from ribosomes (RPFs-translatome) and cell lysates (mRNA- transcriptome) of C57BL/6 mice testes. Different concentrations of RNA (10 µg, 1 µg, 100 ng and 10 ng) were used for the optimization of a commercially available small RNA library prep kit. Data obtained using the standard ribosome profiling protocol (Ingolia et. al 2010) was compared to the data generated by our approach. Finally, in order to estimate the amount RNA present in different germ-cell types, sub-populations were isolated by Fluorescence-Activated Cell Sorting (FACS).
Results: Using this new approach, we generated translatome data from as little as 10 ng of RPFs. We found that, after optimization, we could decrease the amount of time to generate sequencing libraries from three weeks to eight days, without compromising library quality. With our new libraries, we observe 8%-60% reads

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mapping to the mm10 ref genome, with only 4% of those corresponding to rRNA. Our data was comparable to that obtained using the standard ribosome profiling protocol (Pearson correlation of 0.55), with 80% of the reads mapping in 5'-UTR regions and 10% to gene bodies.
Conclusion: We have developed a new approach for the preparation of NGS libraries, optimized to low concentrations of ribosome-protected RNA fragments, thereby reducing the quantity of cells/tissue required to obtain translatome data. This method can be applied to isolated germ cell-types of different stages in order to investigate the complex regulatory network controlling spermatogenesis and may help identifying new biomarkers of male infertility.

74

THE JAG1/NOTCH CANONICAL EFFECTORS HES1 AND HEY1 BIND TO THE GDNF PROXIMAL PROMOTER TO REPRESS ITS TRANSCRIPTION IN SERTOLI CELLS.

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(Presented By: Parag Parekh, PhD)

Introduction: Self-renewal and differentiation of spermatogonial stem cells (SSC) are fundamental processes ensuring normal spermatogenesis throughout life. Within the spermatogonial stem cell niche, GDNF produced by Sertoli cells and peritubular cells plays a major role in the maintenance of a pool of undifferentiated spermatogonia. While GDNF production by Sertoli cells is positively driven by FSH and FGF2, we postulate that negative feedback regulation may also be involved in homeostasis. We previously demonstrated using mouse models and in vitro culture experiments that activation of NOTCH signaling in Sertoli cells reduces their GDNF expression.
Methods: To investigate which cells in the seminiferous epithelium are driving NOTCH in Sertoli cells, we analyzed levels of expression of several NOTCH ligands by different types of germ cells isolated by FACS (GFRA1+, KIT+/DAZL+ and KIT-/DAZL+).
Results: Our data demonstrated high expression of JAG1 by GFRA1-positive germ cells, which decreased as germ cells differentiate. We next investigated whether downstream effectors of NOTCH signaling such as HES/HEY transcriptional repressors directly bind to the GDNF promoter. Our analysis revealed several N- and E-binding sites in the proximal promoter. ChIP-PCR using HEK293 cells and primary Sertoli cells demonstrated specific binding of HES1 and HEY1 to the GDNF promoter, indicative of direct regulation of GDNF transcription by Notch signaling. Further, to verify the functional activity of HES1 and HEY1, we performed Luciferase assays using HEK293 cells co-transfected with a GDNF promoter-luciferase reporter construct and HES1 or HEY1 expression vectors. Our results demonstrate significant down-regulation of Luciferase activity by both HES1 and HEY1 proteins. Altogether, our data conclusively establish that the JAG1/NOTCH/HES/HEY axis down-regulates GDNF expression in Sertoli cells and that GFRA1 positive cells may ensure homeostasis through negative feedback regulation.

75

GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) PROMOTES SPERMATOGENIC REGENERATION AFTER HIGH-DOSE ALKYLATING CHEMOTHERAPY.

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(Presented By: Travis Kotzur)

Introduction: Though chemotherapy and radiation treatments for cancer are lifesaving, a consequence of their use can be a lifetime of side-effects for survivors, including male infertility. Male cancer survivors often present with non-obstructive azoospermia (NOA), and in some cases, this results from a complete loss of the spermatogonial stem cells (SSCs) responsible for ongoing sperm production in adults in a disorder called Sertoli cell-only syndrome (SCO). NOA and SCO in adults can be circumvented by cryopreserving sperm before sterilizing therapies, but approaches for preserving fertility in pre-pubertal male cancer patients who are not yet making sperm remain experimental. Recently, we reported a novel approach in which granulocyte colony-stimulating factor (G-CSF) preserved spermatogenesis after alkylating chemotherapy (busulfan).
Objectives: The objectives of the current study were to resolve the timing during with which G-CSF promotes spermatogenic recovery after busulfan treatment along with the potential responsible mechanisms.
Methods: We treated male C57BL/6 mice with G-CSF (250ug/kg/day, or vehicle) before and/or after busulfan (44mg/kg, or vehicle) treatment and evaluated spermatogenesis histologically 10 weeks later. An additional cohort of mice were similarly treated and immunofluorescent staining for PLZF and Ki67 were performed 1 week after treatment to assess the proliferation index of undifferentiated spermatogonia.
Results: Mice treated with G-CSF after busulfan treatment exhibited a significant increase in the proportion of seminiferous tubule exhibiting spermatogenesis over animals receiving busulfan plus vehicle. Animals receiving G-CSF before busulfan treatment exhibited exacerbated loss of spermatogenesis. Acutely after treatment, we also found a significant increase in proportion of PLZF+ spermatogonia that were Ki67+ in mice that received G-CSF over controls.
Conclusions: These results suggest G-CSF promotes regeneration of spermatogenesis after alkylating insult by stimulating proliferation of surviving spermatogonia. Results of this study clarify the mechanism by which G-CSF protects spermatogenesis after alkylating chemotherapy, suggesting it may be useful as a retrospective fertility-restoring clinical treatment.
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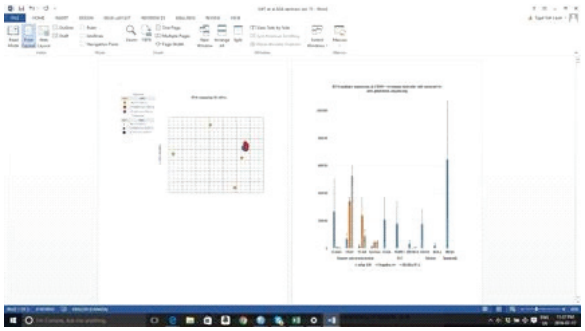
76

EVALUATION OF THE EFFECT OF TWO DIFFERENT CULTURE CONDITIONS ON THE IN VITRO GROWTH OF CD90+VE HUMAN TESTICULAR CELLS USING NEXT GENERATION SEQUENCING (NGS)

Itai Gat, MD[1]; Melissa Felice[1]; Leila Maghen[1]; Ekaterina Shlush[1]; Khaled Zohni[1]; Keith Jarvi[2]; Kirk Lo[2]; Andree Gauthier Fisher[1] and Clifford Librach[1]
[1]CReATe Fertility Centre, Toronto, Ontario, Canada; [2]Urology Deprtment, Mount Sinai Hospital, Toronto, Ontario, Canada
(Presented By: Itai Gat, MD)

Introduction: Successful In vitro spermatogonial stem cell (SSC) expansion is crucial to achieve fertility preservation for pre-pubertal males prior to gonadotoxic exposure. Testicular somatic cells appear to provide a vital support for SSCs in culture. Our aim was to characterize human testicular somatic cells before and after culture with supplemented StemPro34- or DMEM/F12-based culture media. We used CD90, a well-known mesenchymal and somatic cell marker, for sorting prior to characterization in order to establish a homogeneous cell population.
Methods: Six human testicular samples, five from orchiectomies and one from a microTESE were obtained with written informed consent. Each sample was processed by mechanical and 2-step enzymatic digestion to obtain a cell suspension, which was subjected to differential plating (DP) on gelatin-coated plates. After 24 hours, floating cells were removed and attached cells were cultured on laminin-coated plates for two passages (P0 and P1) using StemPro-34 or DMEM/F12- based media. We performed fluorescence activated cell sorting (FACS) using the somatic cell-associated marker CD90 after DP and after P1. CD90+ve cells were characterized by NGS using a custom Ion Ampliseq panel to detect 89 human testicular RNA transcripts. RNA transcript expression after DP and after P1 as well as between media were compared.
Results: The proportion of attached CD90+ cells increased after culture in both StemPro34 and DMEM/F12 conditions (90.1±10.8% and 76.5±17.4%, respectively) compared to after DP (66.3±7%, P>0.05). StemPro34 and DMEM/F12 samples clustered closely on a principle components analysis (PCA) map but non-cultured cells did not. In both media, most of the somatic cell and mesenchymal stromal cell-associated marker transcripts were significantly increased, while germ cell-associated genes were almost eliminated, compared to post-DP samples.
Conclusions: Gene expression profiles of CD90+ve adherent testicular cells changed considerably after culture in both media, but this change was similar between the two culture conditions. These changes suggest that somatic cells with similar mesenchymal stromal cell properties are selectively expanded under both culture conditions.



Monday, April 4, 2016
***Poster Session II**
11:15 a.m. – 12:30 p.m.
Location: Astor Ballroom & Astor Gallery
**Not CME Accredited*

55

IMPACT OF NUMBER OF CYCLES OF COLLAGENASE CLOSTRIDIUM HISTOLYTICUM ON OUTCOMES IN PATIENTS WITH PEYRONIE'S DISEASE

James Anaissie, Faysal A. Yafi, MD, FRCSC; Elizabeth J. Traore; Suresh C. Sikka, PhD, HCLD and Wayne J.G. Hellstrom, MD, FACS
Tulane University
(Presented By: James Anaissie)

Introduction: Intralesional injection of Collagenase Clostridium Histolyticum (CCH) is a minimally invasive, Food and Drug Administration (FDA)-approved, and effective treatment for Peyronie's disease (PD). We sought to analyze the impact of the number of cycles of CCH therapy on outcomes in order to further optimize therapy.
Methods: We conducted a retrospective review at our institution of the records of all patients treated with CCH for PD between 04/2014 and 09/2015. Collected variables included demographics and pre- and post-treatment sexual function, penile curvature, penile vascular findings and treatment outcomes. The primary outcome was significant (≥20%) decrease in penile curvature over baseline at the conclusion of treatment. A repeated measures model was used to determine change in curvature per treatment cycle.
Results: A total of 78 patients were included in the study, of which 36 (46%) completed four cycles of treatment. For all-comers regardless of numbers of cycles, curvature improved from 58.5 degrees pre-treatment to 42.0 degrees post-treatment ($p<0.001$). In the repeated measures model, penile curvature improved significantly following the first three cycles, but not the fourth. Overall, patients who had a ≥20% final reduction in curvature had significantly more response following the first injection (-16.8 degrees vs. -6.2 degrees, $p<0.001$) and underwent more cycles of CCH (3.6 vs. 2.5, $p<0.001$).
Conclusions: CCH therapy is an effective treatment for PD, although the therapeutic benefit may decline after the third cycle of treatment. Patients with ≥20% reduction in curvature over baseline at the conclusion of treatment displayed better curvature improvement after the first cycle and received more cycles of

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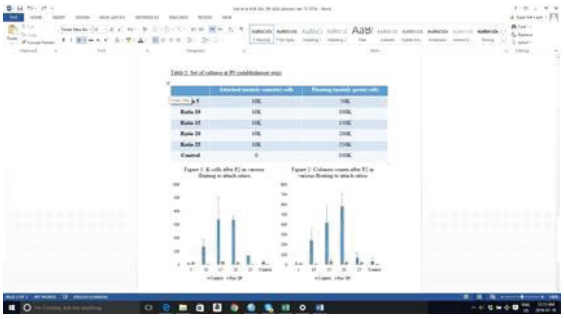
CCH.

77

REPEATED DIFFERENTIAL PLATING RESULTED IN REDUCED MURINE TESTICULAR GERM CELL PROLIFERATION AND COLONY FORMATION

Itai Gat, MD; Leila Maghen; Ekaterina Shlush; Shlomit Kenigsberg; Khaled Zohni; Melissa Filice; Andrée Gauthier-Fisher and Clifford Librach
CReATe Fertility Centre, Toronto, Ontario, Canada
(Presented By: Itai Gat, MD)

Introduction: Spermatogonial stem cells (SSCs) are required for spermatogenesis in males, and hold promise for the development of fertility preservation strategies for pre-pubertal boys having to undergo gonadotoxic cancer treatment. Expansion of SSCs in vitro, to overcome their low frequency in testicular biopsies, is associated with technical challenges, including the overgrowth of somatic cells in culture. Our aim was to explore whether repeated differential plating (DP), a procedure often used to enrich germ cells in primary culture, favors SSC expansion, using a mouse model.
Methods: Testes of adult 6-8 week old CD-1 mice (n=3) were harvested. Mechanical and 2-step enzymatic digestion was conducted to obtain cell suspensions which were incubated on gelatin-coated plates overnight for DP. Following DP, cells at P0 were cultured under five different floating-to-attached cell ratios. Cultures containing only floating cells were used as controls (Table 1). To passages cells from P0 to P1 and P1 to P2, one group of cultures were subjected to recurrent DP to maintain a low density of attached cells (≤10K), while in control cultures DP was not performed (non-DP). Somatic cell and germ cell colonies were counted at each passage and compared between the groups. Total culture duration was 45-50 days.
Results: Recurrent DP resulted in a significant reduction in the total number of cells, as well as germ cell colonies, when compared to non-DP controls. Both the cell and colony counts were significantly higher in the control culture conditions compared to recurrent DP (Figure 1 and 2) (both $p<0.0001$). No differences in total cell number and colonies number between ratios were found between recurrent DP culture conditions, while in the control non-DP group, a significant increase in germ cells was observed at the intermediate 15 and 20 floating-to-attached cell ratios, compared to higher and lower ratios ($p<0.001$).
Conclusions: In conclusion, we found that recurrent DP to restrict murine testicular somatic cell overgrowth does not lead to efficient germ cell colony formation in vitro.



78

SUBTLE BLOOD LOSS CAN BE ASSOCIATED WITH A LACK OF RESPONSE TO TESTOSTERONE REPLACEMENT THERAPY

Phuong Tran, MD; Robert Tan, MD and Patrick Whitfield, NP-C
Low T Institute
(Presented By: Phuong Tran, MD)

Introduction: As testosterone replacement therapy (TRT) becomes more widely accepted, more practitioners will need to be familiar with associated adverse events. We present an unusual case that highlights the need for frequent monitoring of patients on TRT.
Methods: Case Report: A 45-year-old man being treated with injectable TRT for the past year presents for follow-up. Despite an increase in dose three months ago, he noted more fatigue over this time period. He felt that his fatigue had been completely alleviated in the past with therapy. The patient denied any orthopnea, paroxysmal nocturnal dyspnea or weight gain. He denied any abdominal pain, hematochezia or melena.
Results: Physical Exam was unremarkable. Blood pressure: 124/80 Heart rate: 78 Weight: 192 lbs. Lab findings: Total testosterone: 634 ng/dL, Calculated free testosterone: 22 ng/dL, Estrogen: 31 pg/mL, Hemoglobin: 14.5 g/dL (prior Hgb 16.7 g/dL), Hematocrit: 45 % (prior Hct 50.5 %) MCV: 73 fL (prior MCV 85 fL). Upon further questioning, the patient admitted to daily use of diclofenac (a NSAID) for 5-6 months. The patient subsequently underwent upper gastrointestinal endoscopy which revealed gastric ulcers consistent with NSAID use. After cessation of NSAIDs, his follow-up three months later showed a Hemoglobin of 16.1 g/dL and Hematocrit 50.8%, and his fatigue had resolved.
Conclusion: When a patient on TRT develops worsening of previously alleviated symptoms, an increase in dose will often be the first action but may not solve the patient's problem. Careful attention to bloodwork may reveal an alternative diagnosis. In this case, the patient had an occult bleed resulting in a microcytosis yet no anemia, with a seemingly "normal" Hgb/Hct. The stimulatory effects of testosterone on erythropoiesis were able to mask the blood loss by maintaining a "normal" Hgb/Hct level. Testosterone stimulates erythropoietic action in the marrow, and a knowledge of the adverse events related to TRT helped identify an occult bleed in this case. NSAID related GI complications are one of the most common drug-related side effects. With their widespread use, they are seen as safe and often patients fail to mention them as a drug they are taking. This case highlights the clinical importance of follow up on patients on TRT. It also highlights the importance of routine medication reconciliation.

79

MECHANISM OF HYPOGONADISM IN THE TRANSGENIC SICKLE CELL MOUSE

Arthur Burnett, MD; Haolin Chen, PhD; Yuxi Zhang, MD; Terry Brown, PhD; Barry Zirkin, PhD and Biljana Musicki, PhD
Johns Hopkins Hospital
(Presented By: Arthur L. Burnett, II, MD, MBA)

Introduction: Testosterone (T) deficiency is associated with

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sickle cell disease (SCD), but its underlying mechanism is not known. We investigated the possible occurrence and mechanism of T deficiency in a mouse model of human SCD.
Methods: 7 month old homozygote SCD (Sickle) mice were used. Age-matched wild type (WT) and heterozygote SCD (Hemi) mice served as controls. Blood was obtained for measurements of T and luteinizing hormone (LH) by radioimmunoassay (RIA). Testes were collected for Leydig cell isolation, measurements of intratesticular T by RIA, and protein expressions of steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (P450scc), gp91phox subunit of the reactive oxygen species-generating enzyme NADPH oxidase, oxidative stress (4-hydroxy-2-nonenal, 4-HNE), and an antioxidant glutathione peroxidase-1 (GPx1) by Western blot. Leydig cells were treated with LH (0.5 and 10 ng/ml), dibutyryl cAMP (dbcAMP, 1 mM), 22-hydroxycholesterol (22HC, 25 μM), and pregnenolone (P5, 25 μM), and T produced into the media was measured by RIA.
Results: Sickle mice exhibited decreased ($P<0.05$) serum and intratesticular T and increased ($P<0.05$) LH levels compared with WT mice, indicating primary hypogonadism in Sickle mice. LH-, dbcAMP-, and pregnenolone- (but not 22-hydroxycholesterol)-stimulated T production by Leydig cells isolated from the Sickle mouse testis was decreased ($P<0.05$) compared to that of WT mice, implying defective Leydig cell steroidogenesis. There also was reduced ($P<0.05$) protein expression of STAR, but not P450scc, in the Sickle mouse testis. The Sickle mouse testis exhibited upregulated ($P<0.05$) NADPH oxidase subunit gp91phox and increased ($P<0.05$) oxidative stress, and unchanged protein expression of GPx1. Hemi mice exhibited intermediate hypogonadal changes between those of WT and Sickle mice.
Conclusions: Hypogonadism is present in Sickle mice, mimicking the human condition. The defects in Leydig cell steroidogenic pathway, mainly due to reduced availability of cholesterol for T production, may be related to NADPH oxidase-derived oxidative stress.

80

IMPROVING FERTILITY OF BULLS AND BUFFALO-BULLS

Gamal Elamrawi, PhD
Aleaxndria University/Faculty of Veterinary Medicine
(Presented By: Gamal Elamrawi, PhD)

Four native bulls and four buffalo-bulls (7-8 years age) were used for investigating the effect of L-tyrosine on the libido (libido index and reaction time), semen quality and fertilizing capacity. Serum and semen samples were collected for three weeks (twice per week) before treatment to serve as controls. A single oral dose of L-tyrosine (100 mg/kg body weight) was given to each animal.
On collection, libido index and reaction time were measured. Semen was evaluated for ejaculate volume, sperm motility, and percentage of live sperm, sperm cell concentration and sperm abnormalities. Testosterone concentration was assessed in serum using RIA technique.
The result revealed that, a single oral dose of L-Tyrosine leads to improvement in testosterone level, libido, semen quality and sperm fertilizing capacity in bulls.

81

THE INNOVATIVE COMBINATION OF THE HERBAL REMEDY IN INFERTILE COUPLES WITH MALE FACTOR COMPARATIVE SUCCESS OF ASSISTED REPRODUCTIVE TECHNIQUES

Arash Khaki, PhD[1]; Elaheh Uladsaheb Madarek, PhD[2] and Nava Ainehchi, PhD[2]
[1]Department of Pathobiology,Tabriz Branch,Islamic Azad University,Tabriz,Iran.; [2]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
(Presented By: Arash Khaki, DVM, PhD)

Introduction: Nowadays, infertility of men is one of the most common problems in the medical field and the main reason of it is impaired sperm production. Various treatment of infertility in majority of procedures has very high costs. Nowadays using medicinal plants due to the low complication rate and low cost has special significance. In this study, a combination of several plants was evaluated on male infertility.
Methods: In this clinical trial, 40 infertile men of couples randomly during nine months from Al-Zahra Medical Center were selected and for six months treated with a combination of seven plants including: Allium cepa, Zingiber officinale, Ocimum basilicum, Cinnamomum verum, Citrus sinensis' peel, Citrullus lanatus' seeds were placed in capsules of 700 mg (once a day). Before and after treatment, the patients' blood and semen samples were collected to evaluate and analyzed.
Results: Number ($P=0.001$), overall movement ($P=0.002$), Moving forward ($P=0.002$), normal morphology of sperms ($P=0.006$) and patients' blood glucose levels ($P=0.036$) before and after treatment showed significant improvement. The total volume of semen ($P=0.49$), total cholesterol ($P=0.17$), testosterone ($P=0.51$), LH ($P=0.19$) not significantly different with the use of a combination of herbal medicine. Seven couples during the study and after three months of receiving medication (Medicinal herbs combination) were pregnant.
Conclusion: Consumption of medicinal herbs combination with the lowest risk, lowest cost and relatively good effectiveness improve semen and blood sugar of patients significantly. It seems that the mechanism of action is through counteracting the effects of oxidative stress.

82

A ROLE OF KIT RECEPTOR SIGNALING FOR PROLIFERATION AND DIFFERENTIATION OF RAT STEM LEYDIG CELLS

Xiaomin Chen, PhD; Shiwen Liu, PhD; Yiyan Wang, PhD; Linxi Li, PhD; Guiming Wang, PhD; Xiaoheng Li, Master; Haolin Chen, PhD; Jingjing Guo, PhD; Han Lin, PhD; Qingquan Lian, PhD and Renshan Ge, PhD
(Presented By: Xiaomin Chen, PhD)

In the testis, KIT ligand (KITL, also called stem cell factor) is expressed by Sertoli cells and its receptor (c-kit, KIT) is expressed by spermatogonia and Leydig cells. Although KITL-KIT signaling is critical for the spermatogenesis, its roles in Leydig cell development during puberty are not clear. In the

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present study, we investigated effects of KITL on stem Leydig cell proliferation and differentiation. Using an in vitro culture system of seminiferous tubules from Leydig cell-depleted testis, we found that KITL increased the proliferation activity of stem Leydig cells at higher concentration (10 and 100 ng/ml). Low concentration (1 ng/ml) of KITL significantly induced the differentiation of stem Leydig cells via increasing the expression level of steroidogenic acute regulatory protein (Star). In contrast, higher concentration (100 ng/ml) of KITL inhibited the differentiation of stem Leydig cells via inhibiting steroidogenic enzyme (Cyp11a1, Cyp17a1, and Hsd17b3) expression levels. We cultured rat progenitor Leydig cells with KITL for 48 hours and did not find any influence of KITL on the proliferation and androgen production of these cells. In conclusion, KITL is a growth factor that regulates the development of the stem Leydig cell.

83

LONGTERM-TREATMENT OF OVERWEIGHT OR OBESE HYPOGONADAL MEN USING INTRAMUSCULAR TESTOSTERONE UNDECANOATE: LONGTERM-EXPERIENCE OF 4-7 YEARS

Michael Zitzmann, MD, PhD[1] and Farid Saad, MD[2]
[1]University Clinics Muenster; [2]Bayer Inc
(Presented By: Michael Zitzmann, MD, PhD)

Introduction: The intramuscular injection of the long-acting ester testosterone undecanoate (TU) offers a convenient modality for testosterone substitution. The effects of such a therapy on body composition have been well documented but long-term effects and inter-patient variability remain to be elucidated.
Methods: We report data from a total cohort of 453 patients who received treatment by testosterone undecanoate 1000 mg (intramuscular injections) for a treatment time of four to seven years. Of these, 176 men (39%) were overweight (BMI 25-30 kg x m-2) and 235 men (52%) were obese (BMI 25-30 kg x m-2). Increased waist circumference (WC) (94-102 cm) was seen in 99 men (22%) and a substantially increased waist circumference (>102 cm) in 312 men (69%). Age range of men with increased weight/WC was 21 to 68 years (mean 44±11 years).
Results: Individual dosing intervals ranged from 10 to 14 weeks Serum T concentrations increased from an average of 4.7 nmol/L to stable trough levels of 13.3 nmol/L within the first year of treatment and further on to levels between 15 and 16 nmol/l thereafter. Obese patients were not significantly different from in this regard from overweight patients or normal weight patients. The proportion of men fulfilling the new Harmonized Criteria for definition of the Metabolic Syndrome decreased from initially 88% to 65% within the first year and further on to 45% within seven years (Chi-square for trend:$p<0.001$). During the maximal duration of treatment, an overall favourable change from baseline was visible for a multitude of parameters related to androgen effects/metabolic risk, especially lipid parameters (HDL-cholesterol, LDL-cholesterol, triglycerides), blood pressure and fasting glucose levels (all with $p<0.001$ in ANOVAs). Overall loss of weight, WC and also changes in metabolic parameters were log-linear interactions of initial obesity/WC, time and individual increment in testosterone levels. Advancing age was attenuating the effect of weight loss, but not completely. In summary a change of class was seen over time. Eight-two of 176 men changed from overweight to normal weight and 127 of 235 men from obese to overweight.
Conclusion: Intramuscular injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal obese men of a wide age-range, substantiated by a long experience, facilitating a decrement of metabolic/cardiovascular risk factors and fundamental weight loss.

84

CONTEMPORARY TESTOSTERONE PRESCRIBING PATTERNS AT AN ACADEMIC HEALTH CENTER

Jason Jameson, MD
Mayo Clinic Arizona
(Presented By: Jason J. Jameson, MD)

Introduction: Prescriptions for testosterone have increased significantly over the past decade. However, during the past two years, increasing concern for risks of testosterone use as well as an FDA label change noting possible cardiovascular risks may have an effect on testosterone prescribing patterns. The objective of the study was to determine the testosterone prescribing patterns at Mayo Clinic Arizona between 2011 and 2015.
Methods: An electronic medical record database search was performed to identify all men who received a new prescription for testosterone between 2011 and 2015, with various data points identified.
Results: The total number of men receiving a new testosterone prescription decreased linearly from 268 in 2011 to 132 in 2015. Men ages 51-59 accounted for the largest group treated at 49.2%, followed by 24.5% ages 70 and older, 20.5% ages 36-50, and 5.9% ages 18-35. Prescribing Provider Specialty was Primary Care in 47.5%, Endocrinology in 22.1%, Urology in 14.9%, and Other in 15.5%. Primary Care testosterone prescribing decreased linearly during the time period. The type of initial testosterone product prescribed was 74.8% topical, 24.4% injectable, 0.8% implant, and 0.1% buccal. Topical prescriptions decreased linearly during the time period.
Conclusions: The overall number of men receiving testosterone replacement has decreased in the past five years at Mayo Clinic Arizona, possibly related to recent FDA warnings and reports of increased risk, though other unknown factors may have contributed. Primary Care is the largest prescribing specialty with topical agents most commonly used, however both of these factors have decreased during the studied time period.

85

EFFECT OF LPCN 1021 (ORAL TESTOSTERONE) IN HYPOGONADAL MEN REPORTING PSYCHOSOCIAL SYMPTOMS AT BASELINE – SUBGROUP ANALYSIS OF SOAR (STUDY OF ANDROGEN REPLACEMENT) TRIAL

Martin Miner, MD[1]; Christina Wang, MD[2]; Jed Kaminetsky, MD[3]; Adrian Dobs, MD, MHS[4]; Irwin Goldstein, MD[5]; Anthony Delconte, MD[6]; Nachiappan Chidambaram, PhD[7]; Satish Nachaegari, MS[7]; Mahesh Patel, PhD[7] and Mohit Khera, MD[8]
[1]Brown University and Miriam Hospital; [2]Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA; [3]University Urology Associates, New York, NY; [4]Johns

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Hopkins University School of Medicine, Baltimore, MD; [5]Alvarado Hospital, San Diego, CA; [6]Lipocine, Inc. Salt Lake City, UT, and Saint Joseph's University, Philadelphia, PA; [7]Lipocine, Inc. Salt Lake Cit, UT; [8]Baylor College of Medicine, Houston, TX
(Presented By: Martin Miner, MD)

Introduction: Psychosocial symptoms such as depression may be associated with hypogonadism. LPCN 1021 is a novel oral Testosterone (T) undecanoate formulation that may avoid some undesirable attributes of non-oral T, such as risk of transference and skin irritation.
Methods: SOAR was a randomized, active-control, 2-arm, 52-week, open-label, multicenter, dose-titration trial in 314 hypogonadal men (T<300ng/dL on 2 separate days) 18 to 80 years old. Medical history for 98 subjects (31%) included conditions categorized as psychosocial symptoms (PS) such as depression or anxiety. Subjects were randomized to LPCN 1021 (n=210) or Androgel 1.62% (n=104). The LPCN 1021 dose could be titrated up or down at Weeks 4 and 8 based on 24 h pharmacokinetic (e.g. T Cmax and T Cavg24h) profile, if required. Androgel was titrated based on manufacturer's instruction. Sexual function and mood changes were assessed by the Psychosexual Daily Questionnaire (PDQ) for seven days preceding visits. Quality of life (QoL) was assessed by the SF-36 questionnaire at Weeks 1 and 52 (end of study, EOS).
Results: Subjects with and without PS had comparable demographic and baseline T, sex hormone binding globulin, free T, dihydroxytestosterone (DHT), and estradiol (E2) levels. Nine of 10 SF-36 subscores and 5 of 8 PDQ subscores were significantly lower for subjects with PS than without PS at baseline indicating worse sexual function, mood, and QoL. Significant ($p<0.05$) improvement was observed post LPCN 1021 for subjects with PS at the end of study vs baseline in free T (9.7 vs 5.0 ng/dL); SF-36 subscores of vitality, mental health and mental component summary; and PDQ subscores of sexual desire, activity, sexual pleasure with and without partner, erectile function, and positive and negative mood. Significant improvement post Androgel for subjects with PS was observed for free T (9.1 vs 5.6 ng/dL) and PDQ parameters similar to LPCN 1021 except for negative mood; no differences in SF-36 subscores were noted. Subjects with and without PS had comparable mean Cavg24h at Week 13 (efficacy visit) for T (437 vs 488 ng/dL), (110 vs 116 ng/dL) and E2 (29 vs 29 pg/mL) post LPCN 1021 therapy. Subjects with PS significant ($p <0.05$) improvement was observed for bodily pain, general health and negative mood compare to subjects without PS post LPCN 1021 therapy.
Conclusions: Twice daily administration of LPCN 1021 for 52 weeks appeared to improve several QoL and sexual function parameters in subjects with PS.

86

NOVEL MOUSE SEMINIFEROUS TUBULE CULTURE SYSTEM TO INVESTIGATE THE INTERACTION BETWEEN THE STEM LEYDIG CELL AND ITS NICHE

Ren-Shan Ge, MD; Guimin Wang, MD; Linxi Li, PhD; Siwen Liu, MD; Yiyan Wang, MD; Jingjing Guo, MD; Haolin Chen, PhD and Qingquan Lian, PhD
The Second Affiliated Hospital of Wenzhou Medical University
(Presented By: Renshan Ge, MD, MS)

Stem Leydig cells have been demonstrated to differentiate into adult Leydig cells via intermediate stage of progenitor and immature Leydig cells. However, the exact regulatory mechanisms are unclear. We hypothesize that stem Leydig cells are localized on the surface of seminiferous tubule (ST) and are interacted with ST. STs were isolated from adult male mouse and treated with 3 mM ethane dimethane sulfonate (EDS) to eliminate adult Leydig cells on the surface of ST without disturbing stem Leydig cells. EDS-treated STs were then cultured with growth factors including 10 ng/ml platelet-derived growth factor AA, or interleukin 1α or transforming growth factor 1β for 3 days and EDU incorporation was performed to study the proliferation rate of stem Leydig cells. After the treatment of these growth factors, the EDU-incorporated stem Leydig cells were significantly increased by 2-5 folds. Then we tested the differentiation condition for stem Leydig cells into Leydig cell lineage. Mouse STs were cultured in the presence of DMEM/M199 medium (CON), or the medium containing insulin-transferrin-selenium (ITS), 1 ng/ml luteinizing hormone (LH) and both (ITS+LH). After 14 days of culture, the testosterone level was increased to 8.201 ± 2.606 (594- fold), 7.914± 1.018 (573- fold), and 17.950 ± 2.098 ng/well (1301-fold), when compared to control (0.138 ± 0.011 ng/well), indicating ITS and LH alone were enough to induce the differentiation of stem Leydig cells into adult Leydig cells and both factors had synergic effects. Interleukin 1α significantly delayed the differentiation of stem into adult Leydig cells, while platelet-derived growth factor AA stimulates the proliferation of them. In conclusion, platelet-derived growth factor AA primarily stimulates the proliferation of stem Leydig cells, while ITS and LH are synergic to induce them to differentiation.

87

RAT SPERM MRNA TRANSCRIPT LEVELS ARE SENSITIVE INDICATORS OF TESTICULAR DYSFUNCTION

Edward Dere, PhD[1]; Aimee Altemus, BS[2]; James Smith, PhD[2]; Jonathan Phillips, PhD[2]; Kerry Blanchard, PhD[2] and Kim Boekelheide, PhD[1]
[1]Brown University; [2]Boehringer Ingelheim Pharmaceuticals, Inc
(Presented By: Edward Dere, PhD)

The human testis is sensitive to toxicant-induced injury, yet the available tools for detecting exposure effects are limited, insensitive and unreliable. Animal studies use sensitive histopathological endpoints to assess toxicity, but these endpoints require testicular tissue that is not available during human clinical trials. More sensitive and reliable molecular biomarkers of testicular injury that can be used to monitor testicular toxicity in both a clinical and nonclinical setting are needed. In this study, adult male Wistar Han rats exposed for 4 weeks to either cisplatin (0, 0.2, 0.3, or 0.4 mg/kg/day), BI665915 (0, 20, 70, 100 mg/kg/d), BI665636 (0, 20, 100 mg/kg/d) or BI163538 (0, 70, 150, 300 mg/kg/d) to evaluate reproductive toxicity and assess changes in sperm mRNA abundance. None of the compounds resulted in any significant changes in body, testis or epididymis

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weights, nor were there decreases in testicular homogenization resistant spermatid head counts. Histopathological evaluation of the testes found that only BI665915 treatment caused any testicular effects, including minor germ cell loss and disorganization of the seminiferous tubule epithelium, and an increase in the number of retained spermatid heads. A custom PCR-array panel was used to assess changes in sperm mRNA induced by each compound. BI665915 resulted in both significant increases and decreases of each of the mRNAs measured on the PCR array panel; Abi2, Alox15, Gimap4 and Sod3 levels were all elevated by treatment while Clu, Ptgds and Tmeff1 levels were decreased. BI66536 resulted in a significant decrease in Ptgds and Tmeff1 sperm transcript levels relative to vehicle control. These results demonstrate that sperm mRNA levels are sensitive molecular indicators of testicular injury that can potentially be translated into a clinical setting.

88

EXPOSURE TO POLYBROMINATED BIPHENYLS AND SEMEN PARAMETERS, FERTILITY AND ERECTILE FUNCTION IN MEN

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(Presented By: Steven M. Schrader, PhD)

Introduction: Endocrine disruptor chemicals (EDCs) may contribute to the worldwide decline in human sperm quality. Polybrominated biphenyls (PBBs) are EDCs that were tragically introduced into the Michigan food chain in the 1970s, causing state-wide exposure. Contaminated food was quarantined about a year after livestock and humans were widely exposed, however since PBB is lipophilic with a long half-life, thousands more people were exposed in utero and/or during breastfeeding.

Objective: To study the association between PBB exposure and reproductive function in men exposed directly or as offspring.

Methods: We recruited men who were 18 years or older at the time of the study and either born before 1 July 1973 and lived in Michigan sometime during 1973 and 1974 (F0), or born to an F0 parent after 1 July 1973 (F1). Men collected a semen sample and provided information about their fertility and erectile function. We measured current serum levels of PBB153 for F0 and F1 participants. In utero PBB153 levels were also estimated for F1 participants using pre-pregnancy maternal serum PBB levels and a model of PBB-elimination.

Results: Samples were collected from 103 men, 18-57 years old. Current serum PBB153 medians were 0.85 ppb for F0 men and 0.04 ppb for F1 men. For F0 men, PBB153 levels dichotomized at the median were negatively associated with % DNA Fragmentation Index (Beta= -10.67, p=0.055). For F1 men, PBB153 levels as a log-transformed continuous variable were positively associated with sperm viability, both in the univariable and abstinence-adjusted linear models (Beta= 3.83, p=0.015), and negatively associated with moderate to severe erectile dysfunction in a logistic model (OR=0.37, p=0.04). Also for F1 men, estimated in utero PBB153 levels dichotomized at the median were positively associated with elevated sperm concentration (Beta= 0.26, p=0.028); total sperm count (Beta= 0.26, p=0.021); and motility (Beta=21.7, p=0.035); all based on abstinence-adjusted linear models.

Conclusions: Exposure to PBB is associated with unexpected positive effects on semen quality and erectile dysfunction. These associations were found among those directly exposed (F0), as well as those exposed in utero and/or through breastfeeding. These findings are discussed and require further study.

89

INFLUENCE OF AIR POLLUTION OF VEHICULAR ORIGIN IN SEMINAL PARAMETERS OF TRAFFIC CONTROLLERS

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(Presented By: Jorge Hallak, MD, PhD)

Introduction: Population data indicate the Metropolitan Region of Sao Paulo among the 10 most populated cities in the world. With over 10 million inhabitants and, consequently, a large fleet of vehicles, the vehicular air source pollution is a public health problem. Some pollutants are related with alterations in seminal parameters; therefore, the aim of this study was to evaluate the influence of air pollution of vehicular origin on seminal parameters of traffic controllers.

Methods: This prospective study was approved by the Universidade de São Paulo Ethics Committee. Clinical history and urological evaluation were carried out in 61 traffic controllers (exposed group; 27-46 years-old) and 198 fertile men (control group; 22-53 years-old) from the pre-vasectomy group of urologic clinic at Hospital of Clinics of University of Sao Paulo. Were evaluated testicular volume, sexual hormone profile, seminal parameters and tests of sperm function (reactive oxygen species levels and presence of antisperm antibodies). Exposition to air pollution was analyzed through the dispersion of pollutants (NO2, O3 and MP2,5) recorded by Cetesb, generated in the software SURFER 8.0.

Results: The mean of exposure time was 7.3 ± 4.0 years. Traffic controllers presented bigger testicular volume than pre-vasectomy group ($p<0.001$). The exposed group presented progressive motility (27.0 ± 14.0% vs. 40.0 ± 16.2%), sperm morphology by WHO´s criteria (13.0 ± 6.8% vs. 20.0 ± 10.4%) and by Kruger´s strict criteria (3.0 ± 2.5 vs. 6.0 ± 4.3%) diminished than control group ($p>0.001$). Levels of estradiol (p=0.130), LH (p=0.505), FSH (p=0.083) and total testosterone (p=0.447) were at normal range in both groups. Were not observed significant difference in sperm concentration (p=0.343), antibody antisperm (p=0.251) and ROS (p=0.126) between the groups.

Conclusion: Our results suggest that the vehicular pollution

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source has negative influence on sperm quality in traffic controllers in reproductive age, particularly in motility and morphology. Thus, these results indicate that the population of highly polluted areas are susceptible to reduced semen quality and hence male infertility.
Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo.

90

EPIGENETIC AND TRANSGENERATIONAL HEALTH EFFECTS OF EARLY-LIFE PATERNAL EXPOSURE TO ENVIRONMENTAL CONTAMINANTS IN A RAT MODEL

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(Presented By: Janice L. Bailey, PhD)

Introduction: The Arctic food chain is persistently contaminated with organochlorines (OC) so Inuit people have high OC body burdens. There are major health discrepancies between Inuit and non-Aboriginal Canadians, including adverse pregnancy outcomes and congenital anomalies, and their life expectancy 10 years shorter. Although health status is multifactorial, it possible that exposure to OCs contributes to this discrepancy. We hypothesized that early exposure to Arctic OCs affects the paternal epigenome and health of his future generations.
Methods: Sprague-Dawley females (F0) were gavaged with an environmentally-relevant concentration of an Arctic OC mixture or corn oil (Control) and mated to untreated males. Adult F1 males were mated to untreated females to generate F2 litters; F2 development was followed until 90 days of age. F3 and F4 generations were similarly produced. To determine if prenatal OC exposure alters the paternal epigenome, sperm from all generations were analyzed by Reduced Representation Bisulfite Sequencing (RRBS) to obtain genome-wide information on DNA methylation. RRBS libraries were used in paired-end sequencing in 1 lane of a HiSeq 2000 sequencer (Illumina). Analysis and statistics for differentially methylated regions were conducted using Methylkit software. RRBS data were validated by pyrosequencing.
Results: Compared to Controls, F1 and F2 OC-exposed male lineages were subfertile ($P<0.05$), placentas and pups in the F2 litters were smaller ($P<0.05$), and 38% neonatal F2 pups died ($P<0.05$). 13% pups from F3 OC-lineage died preweaning ($P<0.05$). The congenital anomaly, situs inversus, was observed in F2, F3 and F4 OC lineages. RRBS revealed that exposure to the OCs altered F1 sperm DNA methylation, which was confirmed by pyrosequencing. >200 genes in sperm from the OC-exposed F1 males were differentially methylated, including in regions involved in embryo development. Human pathologies linked to the altered genes include metabolic, neurological, psychological and cardiovascular disorders, cancers and reproductive dysfunction, many of which are documented to be related to prenatal exposure to OC or are of concern to Inuit populations. Bioinformatic analysis of the F2-F4 results is underway.
Conclusion: Our results confirm the hypothesis that early-life paternal exposure to environmentally-relevant Arctic OCs induces pathologies in his offspring, possibly due to methylation changes to the sperm DNA.
Funding: Financed by FQRNT & CIHR.

91

LOSS OF TGF?-SIGNALING IN DENDRITIC CELLS LEADS TO EPIDIDYMAL UPREGULATION OF INTERFERON-INDUCED-GTPASES, EPIDIDYMITIS, ANTISPERM ANTIBODIES AND NO ORCHITIS

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(Presented By: Fernando Pierucci-Alves, DVM)

Immunological factors contribute significantly to male infertility and our limited understanding of the pathogenesis of leukocytospermia, epididymitis and antisperm antibodies (ASAs) limits effective therapeutic interventions. Central thymic tolerance is established much earlier than spermatogenesis, and since sperm are immunogenic, the mechanisms of peripheral tolerance are strategic for sperm immunotolerance and for fertility. As we reported previously in abstract format, mice lacking transforming growth factor β (TGFβ)-signaling in dendritic cells (Tgfbr2ΔDC) exhibit epididymal leukocyte infiltrations that reach sperm in the lumen. Epididymal leukocytosis becomes severe, progresses to formation of large sperm granulomas, while the testes exhibit no histopathology. This suggests that Tgfbr2ΔDC males provide a unique model to identify epididymis-specific mechanisms that maintain immunotolerance to sperm, likely via generation of sperm-specific inducible T regulatory cells. In this study, Tgfbr2ΔDC and littermate control males aged 8-10 wks were euthanized and, from each, one epididymis was snap-frozen while the contralateral was subject to histopathology. Total RNA was isoloated from the epididymides of three controls and 3 Tgfbr2ΔDC males with nearly undetectable epididymal leukocytosis and used for microarray analysis (Mouse Gene 2.0 ST; Affymetrix). The Tgfbr2ΔDC epididymis revealed 656 transcripts regulated by a fold change (FC) greater or smaller than + or - 1.5, respectively. Among the 50 genes with greatest upregulation ($2.7 \leq FC \leq 12.0$) were 9 interferon-induced GTPases: guanylate-binding proteins (Gbp) 2, 2b, 3, 4, 7 and 10; immunity-related GTPase family M (Irgm) proteins 1 and 2; and interferon-induced GTPase 1 (a.k.a. as Iigp and Irga6). These data suggest that Tgfbr2ΔDC epididymal DCs synthesize and secrete greater levels of interferon-ɣ, which likely has multiple local pro-inflammatory effects, one of which being local upregulation of interferon-induced GTPases. We are currently investigating whether this is the mechanism by which epididymal DCs may direct or alter the male duct epithelia in response to a danger signal.

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92

?-DEFENSINS PLAY A ROLE IN ANDROGEN-INDUCED WOLFFIAN DUCT MORPHOGENESIS

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(Presented By: Maria Christina W. Avellar, PhD)

Androgens are critical for morphogenesis of the Wolffian duct (WD), the embryonic precursor of the epididymis. Their early effects on WD epithelium are mediated indirectly via mesenchymal paracrine signals by as yet poorly understood mechanisms. We have proposed that β-defensin SPAG11C (sperm associated antigen 11 isoform C) is an androgen-dependent mesenchymal factor required for WD development. SPAG11C expression changes spatio-temporally as the rat epididymis develops from prenatal to postnatal to adulthood. Herein, we investigated the expression of six different β-defensins and their possible androgen modulation during WD morphogenesis. Total RNA was extracted from adult (120-day old) Wistar rat caput epididymis, and WDs at embryonic ages e12.5, e14.5, e17.5 and e20.5. WD (e17.5) organotypic cultures were performed for up to 96 h on cell inserts using serum-free medium with/without testosterone (T, 10 nM) and/or flutamide (10 μM; competitive androgen receptor antagonist) or with purified His-tagged human SPAG11C (hSPAG11C, 80 nM). RT-qPCR studies indicated a differential expression profile of β-defensins in the WD, contrasting with their constitutive expression in the adult caput epididymis (positive control). Spag11c mRNA was detected in WDs between e12.5-e20.5. Transcripts for Defb1, Defb2 and Defb22, however, were only observed in e20.5 WDs; Defb12 and Spag11e mRNAs were not readily detected in any WD analyzed. Defb1 mRNA levels increased, while Spag11c mRNA levels decreased between e17.5 and e20.5, a period when fetal plasma T rises and the WD elongates and coils. Androgen modulation of these two β-defensin transcripts was confirmed by WD organotypic cultures. Gross morphology indicated that hSPAG11C induced a time-dependent impairment of WD elongation and coiling in culture, reducing luminal length, numbers of curvatures and ductal cross-sections, and epithelial cell proliferation rate; all effects were rescued after hSPAG11C washout. Only rare apoptotic cells were detected in both control (T only) and hSPAG11C-treated WDs. The differential expression pattern of β-defensin during androgen-induced development of the WD suggests distinct biological roles and/or specific regulatory mechanisms driving their expression, broadening their relevance to epididymal organogenesis and male fertility.

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93

THE EXPRESSION OF CLAUDIN-1 IN THE HUMAN SPERM IMPROVES EARLY EMBRYO DEVELOPMENT.

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(Presented By: Antonio García Luna Vásquez)

Introduction: Human spermatogenesis is regulated through paracrine and endocrine activity, cell signaling and local control mechanisms. These signaling are nowadays explained by cell-cell interaction. Which implicate spermatogenesis and spermiation involving tight junction proteins that finishes fertilization. Recent preliminary studies suggest that Claudin-1 is involved with sperm and Sertoli cells connections. The goal of this study is to demonstrate the presence of the claudin-1 at human sperm cell and its relation in fertilization process in order to establish sperm functionality.
Objectives: Demonstrate the presence of tight junction (claudin-1) at human sperm in the ejaculated sample of men of infertile couples. Determine the location of expression and its correlation with embryo development.
Methods: Semen samples were obtained by masturbation at the day of the couple's oocyte retrieval, and used for fertilization analysis with WHO 2010 parameters. Claudin-1 expression was evaluated by immunohistochemistry and cytometer. Evaluation of embryo development was conducted by extensive morphological analysis.
Results: We studied ten semen samples of couples with infertility in stimulation cycle; these were divided in two: one for fertilization and the other was marked with Claudin-1 antibodies by immune assay in order to verify the presence of Claudin-1 by cytometer. The sperm cell was evaluated with fluoroscopy for the expression of Claudin-1. Body distribution concentrated primary at the middle piece, with only two of five patients expressing Claudin-1 (40%)(figure 1). Claudin-1 expression correlated with good embryo development. The other three patients that did not express Claudin-1 had bad quality embryos. Our study suggests that the location of Claudin-1 in sperm could interfere with embryo development.
Conclusion: Our results suggest that spermatogenesis and spermiation processes are primordial for obtaining functional sperm cells. Expression of tight junctions markers like Claudin-1 could be the key for the interaction between oocyte and functional sperms. Tight junctions modulate cytoskeletal proteins, contributing with centrosomes migration and correct activation of early embryo development.

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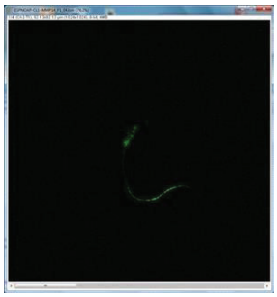

94

SPERMATOGENESIS IN HAMSTER TESTICULAR TISSUE PLACED WITHIN AN ABDOMINAL SCROTUM MICROSURGICALLY CONSTRUCTED IN NUDE RATS

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(Presented By: Fotios Dimitriadis, PhD)

Introduction: We evaluated the spermatogenesis process in hamster testicular tissue (HTT) placed within an abdominal scrotum (AS) constructed in immune deficient nude rats (IDNRs).
Methods: Fragments of HTT recovered from mature hamsters (n=10; 6-week-old) were transplanted into an AS that had been microsurgically constructed in mature IDNRs (n=20; 8-week-old). Just prior to transplantation, hematoxyline eosin stain (HES) in the HTT revealed full spermatogenesis. The AS was constructed in the anterior abdominal wall of each of the IDNRs by removing a circular layer of skin and the respective subcutaneous tissue and by subsequently transplanting/placing a part of the scrotal skin from the respective IDNR in the place where the part of the IDNR-abdominal skin had been removed. The scrotal graft was anastomosed using an operative microscope with the adjacent abdominal skin of the recipient. Thus a part of the abdominal skin was replaced by the scrotal graft and the scrotal graft became the anterior wall of the AS. The posterior wall of the AS was the rectus muscle.
Results: Ten weeks post-transplantation, a part of the transplanted HTT was minced and microscopical observation revealed that within the transplanted HTT the most advanced hamster germ cell was the primary spermatocyte (PS), or the round spermatid (RS), or the spermatozoon in 6, 4, and 4 recipients, respectively. Employment of HES in another fragment of the transplanted HTT demonstrated spermatogenic arrest within the transplanted HTT at the PS stage or the RS stage in 9 or 1 recipients, respectively. HES demonstrated complete spermatogenesis in some tubuli of the transplanted HTT in 4 recipients. In contrast, HES revealed transplanted tissue atrophy in the remaining 6 recipients.
Conclusion: Placement of HTT within a microsurgically constructed AS results in completion of donor meiosis in 40% of the recipients and in completion of donor meiosis plus spermiogenesis in 20 % of the recipient animals. An attractive hypothesis is to autotransplant human testicular tissue within a microsurgically constructed AS in men who are going to receive scrotal or pelvic radiation.

95

ADIPONECTIN REGULATES THE DEVELOPMENT OF RAT STEM LEYDIG CELLS INTO LEYDIG CELL LINEAGE

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(Presented By: Linxi Li, PhD)

The factors affecting the differentiation of rat stem Leydig cells into Leydig cell lineage are largely unidentified. The objective of the present study was to investigate whether adiponectin affects the development of stem Leydig cells. Adult male Sprague Dawley rats were intraperitoneally injected 75 mg/kg of ethane dimethane sulfonate, a chemical that specifically kills Leydig cells. Four days after ethane dimethane sulfonate injection, testis was collected and dispersed as single seminiferous tubules. The seminiferous tubules were cut by 0.5 inch long. The seminiferous tubules were cultured in M199 medium at CO2, 37C for 14 days. The seminiferous tubules were treated by basal Leydig cell differentiation medium. In the medium, different concentrations of adiponectin (1, 10, 100g/ml) were added. The media were collected for measurement of testosterone. 14 days after adiponectin, seminiferous tubules were collected and Leydig cells were stained fluorescently for its biomarker P450 cholesterol side chain cleavage enzyme (CYP11A1). The seminiferous tubules were also harvested for q-PCR and western blotting for different genes of Leydig cell biomarkers. The seminiferous tubules had no Leydig cells on its surface upon isolation. During first 7 days of culture, medium testosterone level was below detectable level. At 14 days, seminiferous tubules from control group produce marginally testosterone. Adiponectin concentration-dependently increased testosterone levels, which were 7 fold higher in 100 ng/ml adiponectin group than that of control. QPCR analysis showed Leydig cell biomarker, Cyp11a1, Cyp17a1, Lhcgr were concentration-dependently increased, while the progenitor Leydig cell biomarker Hsd3b1 levels were without change. CYP11A1-stained Leydig cell numbers were also significantly increased. These results demonstrate that adiponectin is a stimulatory factor for the development of Leydig cells.

96

COMPARISON OF TWO POPULAR VITRIFICATION DEVICES FOR HUMAN IVF

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(Presented By: Tony Tsai, MD)

Introduction: Currently, highly efficient vitrification devices for cryopreservation of human oocytes and embryos play an important role in protecting DNA integrity and consequently IVF pregnancies. Two popular vitrification devices were selected for this study; the results may provide useful guidance for IVF

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laboratories to select the best device.
Methods: 375 consecutive IVF/Donor cycles were included in the study. 1083 mature donor oocytes, 929 cleavage embryos and 695 blastocysts were vitrified 50/50 between cryotop and iVitri®. The survival, fertilization, pregnancy, and miscarriage rates were compared.
Results: There were no significant differences in the survival rates of oocytes (98% vs 99%), cleavage stage (97% vs. 98%) and blastocyst stage (93% vs. 99%) between cryotop and iVitri® devices. However, the increased implantation rate (donor eggs, 85% vs. 70%; cleavage 56% vs. 50%; blastocyst 55% vs.. 48%) and decreased miscarriage rate (donor eggs, 4% vs.7%; cleavage 5% vs. 9%; blastocyst 2% vs. 6%) were found in the iVitri® group as compared to cryotop group.
Conclusion: Our results demonstrate there is a difference between vitrification devices. In our study the iVitri® group had better outcomes. This may be due to the devices better ability to protect DNA integrity, maintain osmolality and freezing speeds.
Funding: The study was partially supported by the Academic Research Project (IIS no. 39252) from Merck and Co. Inc. (T.T /H.L.F).

97

LOSS OF SPERM DNA INTEGRITY AND CHILDHOOD CANCER: IMPACT OF YOGA AND MEDITATION

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(Presented By: Rima Dada, MD, PhD)

Sperm is highly vulnerable to oxidative damage to both nuclear and mitochondrial DNA due to minimal cytosolic anti-oxidants and deficient DNA damage repair mechanism. Recent studies have shown that there is increased incidence of children with Retinoblastoma (Rb) in children conceived by ART and in cases where fathers smoke. This study was planned to analyze the sperm DNA quality in fathers of children with non familial Rb (NFRb).A total of 75 fathers of children with NFRb and 75 fathers of healthy children were recruited for the study. Semen samples were collected and normal semen parameters analyzed. Markers for Sperm DNA damage calculated as DNA fragmentation index (DFI), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and Reactive Oxygen Species (ROS) levels were analysed. The fathers underwent 6 months intervention of yoga and meditation. In the fathers of RB, the seminal mean ROS levels were significantly higher (36.086±1.83 vs 20.51±2.71 RLU/s/million; $p<0.05$) than in controls. There was a significant increase in mean DFI levels (31.50±6.67 vs 21.9±9.4; $p<0.001$) and the levels of 8-OHdG levels (66.02±2.91 vs 23.10±2.71 pg/ml) were significantly higher ($p<0.05$) in fathers of Rb cases vs controls. Only 24 cases underwent 6 month practice of meditation/yoga and these cases showed significant decline in all 3 parameters. Oxidative damage to sperm DNA may be the aetiology of Rb as oxidized mutagenic DNA adducts persist even after fertilization. There was significant improvement in DNA integrity following practice of meditation/yoga and this may reduce incidence of childhood morbidity and even cancer.

98

CYTOGENETIC ABNORMALITIES IN INFERTILE MALE WITH NON-OBSTRUCTIVE AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA

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(Presented By: Moustafa Elsaied, MD)

Introduction: Approximately 15–20% of azoospermic and 5–10% of oligozoospermic infertile males have genetic abnormalities. Chromosomal abnormalities and AZF genes' deletions are important for diagnosis and prognosis of male infertility.
Objectives: To estimate the frequency and types of both chromosomal abnormalities and AZF microdeletions among patients with non-obstructive azoospermia(NOA) and severe oligozoospermia (OS).The exclusion criteria were patients with past or present history of testicular malignancy, radiotherapy or chemotherapy, varicocele, trauma, mumps orchitis, testicular maldescent and orchidectomy.
Methods: This study was conducted on 1127 infertile Egyptian patients including 653 NOA and 474 severe OS patients (sperm count less than 5 million/ml). Chromosomal culture was performed for all patients and AZF microdeletions were tested for 653 NOA and 158 severe OS patients.
Results: The frequency of chromosomal abnormalities was 162/1127 (14.3%); 22.6% in NOA and 3.7% in severe OS. Numerical chromosomal abnormalities were higher than structural type (11.8% vs 2.4%). Klinefelter syndrome (KS) represented the most common chromosomal abnormality (11.2%) while 46, XX male, 47, XYY and marker chromosome represented 0.4%, 0.2%, 0.2% respectively. Chromosomal translocations were (1.8%) and chromosomal inversions (0.5%). Sex chromosomal (gonosomal) abnormalities incidence was higher than autosomal type 11.9% (135/1127) vs 1.8% (21/1127). Gonosomal abnormalities were higher in NOA than in severe OS (20.5% vs 0.2%) while autosomal abnormalities were higher in severe OS than in NOA (2.9% vs 1.1%). KS represented 94.1% (127/135) of gonosomal abnormalities whereas chromosomal translocation was the most frequent autosomal abnormality (16/21=76.1%). AZF microdeletions were found in 5.5% (45/811), higher in NOA than in severe OS (6.1% vs 3.16%). AZFc deletions represented the most frequent finding 68.8% (31/45), followed by AZFbc 15.5% (7/45), AZFb 8.8% (4/45) and AZFa 6.6% (3/45). All patients with AZFa, b and bc deletions were NOA, while 83.3% (26/31) of men with isolated AZFc deletion were NOA and 16.2% (5/31) were severe OS.
Conclusion: The results of our study support the importance of

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karyotyping and AZF microdeletions analysis in NOA and sever OS and help counseling for such patients before their referral to assisted reproductive techniques.

99

DISCOVERY OF SEVERE OLIGOZOOSPERMIA BIOMARKERS VIA TRANSCRIPTOME ANALYSIS IN MATURE SPERM

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(Presented By: Seyedmehdi Nourashrafeddin)

Introduction: Infertility affects nearly 7% of males. Severe oligozoospermia (SO) is the most common semen abnormality observed in infertile men. Yet, the majority of oligozoospermic males are diagnosed with idiopathic infertility. Currently, the molecular origin of SO is poorly understood. Thus, identification of genes with abnormal expression will improve our understanding of SO and bring new valuable biomarkers for the diagnosis of infertile males.
Objectives: Mature human sperm contain a complex set of RNAs that have been implicated in spermatogenesis, fertilization, and early embryonic development. While recent studies have investigated the sperm transcriptome, none have been SO-specific. Next-generation sequencing of sperm RNAs can provide an objective measure of impaired spermatogenesis by revealing misregulated protein coding transcripts in SO sperm. We propose that certain sperm-specific genes, differentially expressed in SO, may be involved in male infertility and could act as informative diagnostic biomarkers for spermatogenetic failure.
Methods: Semen samples were collected from patients with idiopathic SO at the Magee Womens Hospital. Mature sperm was isolated with a swim-up protocol. Total RNA was sequenced using an Illumina platform. To identify transcripts with differential expression, the resulting SO transcriptome was compared against the transcriptome of mature normozoospermic (NO) sperm.
Results: A total of 19,110 transcripts expressed in SO samples were mapped to GRCh38 human reference genome. Preliminary comparative analysis of gene expression profiles in SO resulted in the selection of 2,228 transcripts with significant differential expression. 224 of these transcripts were testis-specific, with 70% down-regulated (n=158) and 30% up-regulated (n=66). Additionally, 58 testis-specific genes were missing in SO and 44 transcripts that are not observed in NO newly appeared in SO. Gene ontology and pathway analysis demonstrated global down-regulation of postmeiotic genes, as well as the perturbation of several critical processes, such as apoptosis and the mitochondrial respiratory chain, that are consistent with reduced sperm count.
Conclusion: We identified a set of differentially expressed genes specific to severe oligozoospermia, including novel and known testis-specific genes. These transcripts could be potential biomarkers for severe oligozoospermia and improve diagnosis and treatment of the disorder.

100

MULTIPLE UNIQUE GENOMIC MUTATIONS FOUND IN AZOOSPERMIC AND OLIGOZOOSPERMIC INFERTILE MALES

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MWRI
(Presented By: Alexander Yatsenko, MD, PhD)

Introduction: Non-obstructive azoospermia (NA) and severe oligozoospermia (SO) are the two most common semen abnormalities found in infertile males. Currently, the cause of non-obstructive azoospermia and severe oligozoospermia is largely unknown in the majority of infertile men and clinical genetic testing is limited. To investigate the genetic causes of NA and SO we carried out a systemic genome-wide study via array CGH aberrations analysis and Whole Exome Sequencing (WES). We screened a population of 100 NA and SO males for submicroscopic aberrations and point mutations in germ-cell candidate genes.
Methods: To detect genomic aberrations we performed 400K whole-genome array comparative genomic hybridization (Agilent) in 100 azoospermic and oligozoospermic males. Similarly, we carried out mutation screening of all coding genes via Whole exome sequencing (Illumina) in 100 patients. Found mutations were checked against multiple polymorphic databases.
Results: Among several rare and unique CGH aberrations detected, we selected 1 found in 2 NA patients; genomic deletion on chromosome Xq13.2 located inside of TEX11. The testis-expressed gene 11 encodes a protein critical for male germ cell meiotic DNA recombination. Tex11-knockout male mice exhibit meiotic arrest (MA) at the pachytene stage. The deletion is 99-kb long and involves 3 exons of TEX11. The loss causes a 79–amino acid in-frame deletion within the protein meiosis-specific domain SPO22. Subsequent mutation screening found 5 novel mutations in 287 AZ patients (total 7/289, 2.4%). Importantly, 5/33 (15.2%) of mutations were found in patients diagnosed with NA and meiotic arrest, reminiscent of the Tex11-deficient mouse phenotype. Preliminary WES analysis identified multiple novel mutations in testis-specific gene candidates in families with NA and SO. The study of random patients can be equally informative when a large population is combined with an effective analytical approach.
Conclusion: The combination of genomic aCGH and WES is more efficient than tactics used in previous studies. We found that hemizygous TEX11 mutations on X chromosome are a frequent cause of meiotic arrest in infertile NA men. Further studies are required to elucidate the heterogeneous causes of SO.

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102

EXPRESSION PATTERN OF RPS6, RBM9 AND RPL10A IN RECURRENT PREGNANCY LOSS

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(Presented By: Vidhu Dhawan, MBBS, MD)

Introduction: Recurrent pregnancy loss (RPL), affecting 0.4-1% of couples is defined as having at least three consecutive embryo miscarriages before 20 weeks of gestation. With increasing literature to explore the causes and consequences of recurrent pregnancy losses, much less has been written on ongoing debate related to the implications of paternal factors in fertilization and embryonic development. Studies have been conducted to assess the role of spermatozoal RNAs from transcriptionally inert mature human spermatozoa which are delivered to the human oocytes at fertilisation.The present study was designed to assess the expression pattern of spermatozoal RPS6 (Ribosomal protein S6), RBM9 (RNA binding motif protein 9) and RPL10A (Ribosomal protein L10A) genes in male partners of couples experiencing RPL. Seminal oxidative stress and DNA Fragmentation Index (DFI) was also assessed.
Methods: Ejaculates were obtained from 30 male partners of couple experiencing RPL and 20 healthy volunteers with proven fertility. Semen analysis was assessed by WHO (2010) criteria. RNA was isolated from semen samples, reverse transcribed and investigated by q-PCR analysis. The relative quantification of target genes was calculated with 2DDCt method after normalization to β-actin. Reactive oxygen species (ROS) levels were assessed by luminol-dependant chemiluminescence. The Sperm chromatin structure assay (SCSA) was performed by flow cytometry to determine DNA fragmentation Index (DFI).
Results: No significant difference was observed in age, seminal volume, liquefaction time, pH and sperm concentration between the male partner of RPL cases and the controls. RPS6, RBM9 and RPL10A genes in RPL cases were seen to show an axis fold change of 1.01, 1.68 and 1.51 respectively with respect to the controls. The mean ROS level in the cases was seen to be higher 603.1 (>25) with respect to controls (22.07). The mean DFI of male partners of couples with RPL (38.29) was significantly ($P < .0001$) higher as compared to controls (23.5). The DFI in all the patients was seen to be >28 against 6% of fertile controls ($P < .0001$).
Conclusion: Spermatozoal RPS6, RBM9 and RPL10A genes have been associated with an increased incidence of miscarriages. The dysregulation in spermatozoal mRNA transcripts may help to explore the diagnostic potential of paternal factors and can be used as biomarkers for discriminating those samples with the worst ability to fertilize oocyte.

103

THE CAENORHABDITIS ELEGANS ORTHOLOG OF HUMAN T-COMPLEX PROTEIN 11 (TCP11), M056.2, IS NECESSARY FOR MALE FERTILITY

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(Presented By: Matthew R. Marcello, PhD)

Human t-complex protein 11 (TCP11) is a testis-specific gene product that is hypothesized to be necessary for proper sperm capacitation, acrosome reaction, and sperm morphology. M05D6.2 is the Caenorhabditis elegans ortholog for human TCP11. C. elegans have two sexes: hermaphrodite and male. Sperm from hermaphrodites and males must undergo proper sperm activation, which includes processes similar to sperm capacitation and acrosome reaction in mammals, to migrate to and fertilize the egg. We have used RNA interference (RNAi) to disrupt the gene function of M05D6.2 in C. elegans. Hermaphrodites subject to M05D6.2 RNAi-treatment show know reduction in fertility. However, when male C. elegans are subject to M05D6.2 RNAi-treatment our preliminary results indicate that they have a significant decrease in fertility. We are now generating animals lacking functional M05D6.2 and animals mimicking single nucleotide variants (SNVs) of TCP11. Together these studies will provide insight into the conserved biological role of TCP11 and provide a platform to investigate the consequences of SNVs on the cellular function of TCP11.

104

POST HOC ASSESSMENT OF SOMATIC CELL CONTAMINATION IN SPERM EPIGENETIC STUDIES USING DNA METHYLATION ANALYSIS OF THE DLK1 DMR

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(Presented By: Tim Jenkins, PhD)

Introduction: The epigenome is a unique set of chemical modifications that facilitates appropriate gene expression profiles. Because each cell is defined by different patterns of transcription, the study of epigenetics is typically performed only on a single tissue or cell type and contamination with other cells is of major concern as this may bias results. This is particularly true in the study of the male germ line as mammalian sperm have extremely unique epigenetic signatures. In addition, because the sperm are haploid cells, each somatic cell that contaminates a sample is approximately twice as influential as a single sperm. Great effort is applied in the study of sperm epigenetics to remove potentially contaminating somatic cells, but a confirmation of the removal of somatic cell DNA is warranted to enable appropriate interpretation of results. Our objective was to detect somatic cell contamination in samples that have undergone array or next generation sequencing analysis.
Methods: Paired samples (sperm and whole blood) from 4 individuals was utilized in this experiment. Various combinations of sperm and white blood cells were screened to identify levels of somatic cell contamination. Two distinct somatic cell lysis protocols (modest and robust) were employed as well to remove

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somatic cells prior to DNA extraction. Methylation signatures were measured on the 450k methylation array (Illumina).
Results: Our analysis revealed distinct significant differences in DNA methylation at the DLK1 DMR (chr14:101191893-101192913) between sperm a somatic cells ($p<0.0001$) with an average fraction methylation of approximately 0.1 and 0.8 respectively. We demonstrated that methylation signatures at this site are altered in direct proportion to the level of somatic cell contamination. Further, we determined that low volume somatic cell lysis protocols are insufficient to remove all potentially contaminating somatic cell DNA from the final analysis even in cases where no somatic cells were visible.
Conclusion: Our data suggest two key findings. First that the DLK1 DMR can offer a simple and informative post hoc assessment of somatic cell contamination that is suitable when performing next generation sequencing or CpG resolution micro array analysis. Second we found that low volume somatic cell lysis protocols are insufficient to remove all potentially contaminating somatic cells or somatic cell DNA when performing sperm epigenetic analysis.

105

EFFECT OF DIFFERENT VITRIFICATION DEVICES ON OOCYTE DNA INTEGRITY IN BOVINE OOCYTES

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(Presented By: Huailiang Feng, PhD)

Introduction: In our previous study, we showed a clinical improvement in Human IVF outcomes with iVitri® versus cryotop. The devices ability to protect DNA integrity, may be the reason for this difference. This study evaluated the DNA integrity between 3 different devices.
Methods: Total of 3000 bovine cumulus–oocyte complexes (COCs) were commercially purchased and matured in vitro for 24 hours at 38.5oC in an atmosphere of 5% CO2 in air. 1600 matured bovine oocytes were selected for vitrification by using commercial cryoloops; cryoleaf, cryotop and iVitri. The survival rate, DNA apoptosis and DNA methylation were assessed after vitrified bovine oocytes were thawed.
Results: The study results demonstrated that there were no significant differences in the survival rates (9.1%, 89.4% and 92.2%) of bovine oocyte with loading vitrification fluid under 0.5µl by using Cryoleaf, Cryotop and iVitri. However, the survival rates (98.2% and 98.5%) were higher in iVitri in the 1.0 µl and 1.5 µl fluid as compared with Cryoleaf (92.2% and 94.2) and Cryotop (90.2% and 89.5%). Furthermore, there was a lower DNA apoptosis (8.3%) and DNA methylation (2.2%) in iVitri comparing with Cryoloop (44.9% and 11.0%), Cryoleaf (34.2% and 7.2%), and Cryotop (29.1% and 5.1).
Conclusion: Our results demonstrate that the type of vitrification device and loading volumes may play an important role in reducing DNA apoptosis and methylation. This may be one reason why iVitri® was superior to the cryotop in our human IVF study.
Funding: The study was partially supported by NSFC abroad young scientist fund (31128013/C120205) (H.L.F) and the Academic Research Project (IIS no. 39252) from Merck and Co. Inc. (T.T/ H.L.F).

106

VARIATION OF DNA FRAGMENTATION LEVELS DURING DENSITY GRADIENT SPERM SELECTION FOR ASSISTED REPRODUCTION TECHNIQUES: A NEW MALE PREDICTIVE PARAMETER OF PREGNANCY?

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(Presented By: Elisabetta Baldi, PhD)

Predicting the outcome of IVF/ICSI is one main goal of current research on assisted reproduction. To understand whether density gradient centrifugation (DGC), used to select sperm, can affect sperm DNA integrity and impact on pregnancy rate (PR), we prospectively evaluated sperm DNA fragmentation (sDF) by TUNEL/PI, before and after DGC. SDF was studied in a cohort of 90 infertile couples the same day of IVF/ICSI treatment. sDF increased in 41 samples (Group A, median sDF value: 29.25% [IQR:16.01-41.63] in pre- and 60.40% [IQR: 32.92-93.53] in post-DGC) and decreased in 49 (Group B, median sDF value: 18.84% [IQR:13.70-35.47] in pre- and 8.98% [IQR:6.24-15.58] in post-DGC). PR was 17.1% and 34.4% respectively in Group A and B (OR: 2.58, 95%CI: 0.95-7.04, p=0.056). After adjustment for female factor and female and male age, the estimated OR increased to 3.17, 95%CI:1.08-9.32, p=0.029). According to the subgroup analysis for presence/absence of female factor, heterogeneity in the association between the Group A and B and PR emerged (OR: 4.22, 95%CI:1.16-15.30 and 1.53, 95%CI:0.23-10.40, respectively for couples without, n=59, and with, n=31, female factor).
This study provides the first evidence that DGC procedure produces an increase of sDF in about half of the subjects undergoing IVF/ICSI, who next show a much lower probability of pregnancy, raising concerns about the safeness of this selection procedure. Evaluation of sDF before and after DGC configures as a new prognostic parameter of pregnancy outcome in IVF/ICSI. Alternative sperm selection strategies for those subjects undergoing the damage after DGC are recommended.

107

A MODIFIED MICROSCOPIC VASOEPIDIDYMOSTOMY TO TREAT EPIDIDYMAL OBSTRUCTION

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(Presented By: Hongtao Jiang, Sr., MD)

Introduction: The aim of this study was to improve the outcomes of microscopic vasoepididymostomy for epididymal obstruction.
Methods: We retrospectively analyze the outcome of surgical treatment of 68 cases of epididymal obstructive azoospermia in our single center between January 2009 and May 2015. All

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patients were enrolled by clinic criteria and underwent a modified vasoepididymostomy. The mean postoperative follow-up time was 22 months. All 68 patients with epididymal obstructive azoospermia were underwent the modified microscopic vasoepididymostomy, 41 bilaterally and 17 unilaterally. In our study, the vasoepididymostomy is similar to transverse two suture intussusception vasoepididymostomy currently used, but the modifications were that we used microsurgical curved scissors to create a round-window tubulotomy between two transverse sutures on the epididymal tubule and the sutures placed in the vas deferens were full thickness (Figure). Semen analyses were initiated at 4 weeks postoperatively, followed by trimonthly (month 3, 6, 9, 12). Patency was defined as more than 10000 sperm per ml of semen. Semen analyses until no sperm was found at 12 months or pregnancy was achieved.

Results: Our study have shown that the patency rate of bilateral VE was up to 92.7% (38/41), and the unilateral patency rate is 70.6% (12/17).

Conclusion: Using modified microscopic vasoepididymostomy technique, a favorable patency can be achieved for epididymal obstructive azoospermia.

108

EFFECT OF YOGA ON QUALITY OF LIFE AND SEMINAL QUALITY IN MALE INFERTILITY PATIENTS WITH MAJOR DEPRESSIVE DISORDER (MDD): RANDOMIZED CONTROLLED TRIAL.

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(Presented By: Madhuri Rajaneesh Tolahunase, BAMS, MSc[Med])

Introduction: Infertile men diagnosed with major depressive disorder (MDD) are at risk for poor quality of life and poor sperm quality due to cellular aging. Targeted interventions are needed to address the needs of this vulnerable group.

Methods: This trial of a short term yoga intervention for infertile men was designed to evaluate improvement of sperm quality (DNA fragmentation Index (DFI), reactive oxygen species (ROS) and total anti-oxidant capacity (TAC)) as a primary outcome. Secondary outcomes included improvement in quality of life (WHOQOL-BREF scale) and reduction in depression severity (Beck Depression Inventory–II (BDI-II)), cellular aging and neurodegeneration (cortisol, ROS, TAC, telomere length, and brain derived neurotrophic factor (BDNF)). Infertile men with MDD were randomly assigned to a 12-week yoga intervention group (n = 27) or to a wait-list control group (n = 22). Participants were examined and semen and blood samples were collected before and after the intervention for assessment. Basic semen analysis based on WHO (2010) guidelines was also performed.

Results: In linear mixed models, the yoga intervention led to significant improvements in basic semen analysis based on WHO (2010) guidelines and significant reductions in sperm DFI ($p = .004$). Compared to controls, the ROS levels were lower in the yoga group ($p = 0.006$), whereas the TAC levels were higher in the yoga group ($p = 0.001$). Yoga group showed significant reductions in depressive symptoms ($p = .044$) and improvement in quality of life ($p=.022$), as well as significant reductions in cortisol, blood ROS and increase in other circulating biomarkers, which include, telomere length, TAC and BDNF at post intervention ($p < .05$ for all).

Conclusion: A brief, yoga intervention demonstrated preliminary short-term efficacy in improving sperm quality. Components of yoga, including asanas (physical postures), dhyana (meditation) and pranayama (breathing exercises), improve quality of life, which brings about decreases in both psychological and oxidative stress. The resulting reduction in depression severity, and improvement in biomarkers of cellular aging and neurodegeneration in males suffering from major depression influences improvement in quality of semen and sperm. Further research is needed to analyze effects of yoga on infertile couples with MDD for benefits in fertility.

109

EFFECT OF IN VITRO VITAMIN E (ALPHA-TOCOPHEROL) SUPPLEMENTATION IN HUMAN SPERM SUBMITTED TO OXIDATIVE STRESS

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(Presented By: Luana Gallego Adami)

Introduction: A male factor is diagnosed in 50% of the cases of infertility. Among the factors that can lead to male infertility, oxidative stress (OS) is the main cause, whose arises as a result of an increase in reactive oxygen species (ROS) and/or a decrease of antioxidants in the seminal plasma. Antioxidants present in seminal plasma balance ROS activity, avoiding possible damages to sperm. However, supplementation with exogenous antioxidants, such as vitamin E, may decrease damages due to excessive ROS in semen. In an attempt to understand the importance of ROS and antioxidants on sperm, the aim of this study was to evaluate the antioxidant effect of in vitro vitamin E (alpha-tocopherol) in human sperm incubated with an inducer of oxidative stress.

Methods: For this study, 32 patients collected semen, which, after semen analysis, was divided into four aliquots: Control (C) incubated with only BWW, Induced (I) incubated with hydrogen peroxide (H2O2, 5mM), Antioxidant (A) incubated with vitamin E (40μM), and a group with both incubations, in the concentrations above (AI). After the incubations, the following sperm functional analyses were performed: (i) intracellular superoxide anion (O2-) activity (dihydroethidium), (ii) acrosome integrity (PNA-FITC), mitochondrial activity (3,3' diaminobenzidine) and DNA fragmentation (alkaline Comet assay). Groups were compared using a Friedman test with a Bonferroni post hoc test, considering $\alpha = 5\%$.

Results: Results are presented in table 1. Incubation with H2O2 led to a decrease in acrosome integrity, mitochondrial activity and an increase in DNA fragmentation and O2- activity when compared to controls. Co-incubation with H2O2 and Vitamin E decreased intracellular superoxide anion activity and increased

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acrosome integrity.
Conclusion: Supplementation of Vitamin E is able to counteract exogenous oxidant activity, which in turn may assist in balancing samples with high oxidative status.
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Table 1. Statistical analysis performed at all groups. Median showed in % (p<0,05).

	C (n=32)	I (n=32)	A (n=32)	AI (n=32)	p
PNA (% acrosomes intact)	89,5; 9,5 [a]	75; 21,9[b]	87,2; 13[a]	93; 13[c]	0,001
DHE (% positives spermatozoa)	10; 12,1[a]	14; 14,5 [b]	10; 13,2 [a]	10; 9,7 [a]	0,001
DAB I (% mitochondria 100% active)	6; 4,7[a]	0; 1,7[b]	5; 6 [a]	0; 2,7 [b]	0,001
DAB II (% mitochondria>50% active)	67,8;24,2[a]	20,5; 31,2[b]	61,5; 31[a]	21,5; 18,2[b]	0,001
DAB III (%mitochondria<50% active)	23,5; 23,7[a]	56,2; 29,5[b]	30; 31,5 [a]	66; 36[b]	0,001
DAB IV (% mitochondria 100% inactive)	0; 2[a]	9; 15[b]	0,5; 3[a]	6; 10,75[ab]	0,001
Comet I (%)	5,5 ; 10[a]	1,5 ; 3[b]	4 ;7,5[a]	3 ; 6[b]	0,001
Comet II (%)	73,5;12,2[a]	61;23,5 [b]	79; 21,7[a]	70; 19,9[b]	0,001
Comet III (%)	16; 12[a]	29,5;17,2 [b]	14,5;22,2[a]	22;20[b]	0,001
Comet IV (%)	1;4,7[a]	4,5; 7,8[b]	1;3,7[a]	2,5; 8,3[ab]	0,001

*amounts expressed in median; interquartile range (IR). Friedman test, Bonferroni post hoc test. On the same line values with the same letter are not statistically different between themselves (p>0,05).C= control group; I= group with hydrogen peroxide; A= group with vitamin E; AI= group with both them. PNA= functional test evaluation of sperm acrosome; DHE= functional test of the presence or absence of the superoxide anion; DAB= functional test of mitochondrial activity mitochondrial; and Comet=

110

OPTIMIZATION OF A HIGH-THROUGHPUT SCREENING (HTS) COMPATIBLE ASSAY FOR THE SEARCH OF POTENT AND SPECIFIC SMALL-MOLECULE INHIBITORS OF THE TESTIS-SPECIFIC SERINE KINASE 1 (TSSK1) ACTIVITY

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(Presented By: Ana Maria Salicioni, PhD)

Introduction: Despite the availability of a range of contraceptive methods, over 50% of pregnancies are unintended worldwide. Male contraception remains an important area of research. Several non-hormonal targets for a male-based contraceptive pill have been brought to light recently, including the testis-specific serine kinase (TSSK) family. This protein kinase family is composed of six members, TSSK1 through TSSK6, yet little is known about their biochemical regulation and function. The finding that TSSK family members are postmeiotically expressed in male germ cells, together with the infertile phenotype found in males of both Tssk1/2 and Tssk6 mouse knock-out models, has led to the proposal that TSSKs play an important role in sperm function, making them excellent targets for drug design of novel male contraceptives. Our research is aimed 1) to gain further insight into the biochemical regulation of this family of protein kinases and, 2) to design a homogeneous in vitro assay compatible for high-throughput screening (HTS) that would allow for the search of potent and specific small-molecule inhibitors of TSSK1 activity.
Methods and Results: In this study, we have optimized a homogeneous, non-radioactive enzyme activity assay to detect rTSSK1 activity. In addition, we have performed a mid-throughput screening of kinase inhibitors and we found some inhibitors that have proven to greatly reduce the activity of TSSK1 overall.
Conclusion: Altogether, our research will contribute to the understanding of the regulation of TSSKs and would allow for the future application of HTS in order to find potent and specific inhibitors suitable for the development of a non-hormonal, reversible male contraceptive.

111

TESTICULAR TISSUE PRESERVATION IN PRE-PUBERTAL AND POST-PUBERTAL MALES

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(Presented By: Nathalie Rives)

The strategy used for male fertility preservation will depend on sexual maturity. In pre-pubertal boys, even if testicular tissue preservation (TTP) has been described as a potential option, this procedure has been exceptionally reported in a human application. We reported the results of the management of TTP at Rouen University Hospital to evaluate the feasibility of testicular tissue preservation, the efficiency of this procedure, the quality of pre-freeze and post-thaw testicular tissue.
All the pre- and post-pubertal boys addressed for TTP between January 2008 and December 2015 were included in our study. Age, urogenital history, clinical and biological data were assessed for each patient. An accurate histological diagnosis was also determined in case of malignant disease. An informed consent for TTP was signed by the parents and the patient if possible, according to the current French law. Each patient underwent a bilateral testicular biopsy. Testicular tissue was frozen using a controlled slow freezing protocol without seeding and a programmable freezer (Milazzo et al., 2008). Integrity and structural modifications of pre-freeze and post-thaw testicular tissue were evaluated semi-quantitatively on paraffin-embedded tissue sections.
A total of 102 patients, aged between 6 months and 36 years were included in our study: Malignant disease accounted for 72% of patients with 35% of acute leukemia. Patients less than 20 years with malignant disease underwent bone narrow transplantation after TTP. TTP was proposed in post-pubertal boys because of sperm cryopreservation failure or severe sperm alterations. Non-malignant disease interested Klinefelter syndrome (7%), hematological disease (10%), and others (10%). Only one hematoma occurred after TTP. After thawing, nuclear and epithelial alterations of seminiferous tubules remained very low. Spermatogonia concentration was significantly decreased in patients who previously received alkylating agents. Interstitial tissue fibrosis and thick basal lamina were more frequently observed in myeloblastic acute leukemia.
TTP is a feasible procedure for fertility preservation both in pre-and post-pubertal males. A slow freezing protocol maintains a good architecture of seminiferous cords. However, the treatment received before TTP even if considered as a low level toxicity may have a negative impact on spermatogonia number and survival.

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ABSTRACTS

112

OPTIMIZATION OF HORMONAL MODULATION FOR SPERMATOGENIC RECOVERY BY SPERMATOGONIAL STEM CELL TRANSPLANTATION

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Cytotoxic treatments such as irradiation in high doses impair spermatogenesis and cause virtually permanent sterility in male mammals due to the loss of spermatogonial stem cells (SSCs). In such cases, in animal models, spermatogenesis could be qualitatively restored from transplanted SSCs. Our previous studies showed that transient hormonal suppression treatment of the recipient with a gonadotropin-releasing hormone antagonist (GnRH-ant), given immediately after irradiation, enhanced spermatogenesis from transplanted SSCs in mice and monkeys. To further optimize hormone suppression regimens, we analyzed spermatogenesis in irradiated (13.5 Gy) C57BL/6 mice that were given the following hormone suppression regimens: (a) GnRH-antagonist (GnRH-ant), (b) GnRH-ant + Flutamide (androgen receptor antagonist), or (c) GnRH-ant + human recombinant FSH prior to transplantation of syngeneic GFP-marked testis cells from pubertal mice. The GnRH-ant treatment was started 5 weeks after irradiation when the germ cell depletion was complete and given for 4 weeks, at the end of which SSC transplantation was performed. Flutamide treatment was started 2 weeks before transplantation and continued for another 4 days. FSH treatment was started one day before transplantation and maintained for 2 weeks. Analyses of endogenous spermatogenic recovery showed slight increases in recovery in all the hormone-modulated groups compared to irradiated-only mice, but the differences were not statistically significant. In contrast, only 1.6% of the tubules showed differentiated germ cells originated from donor spermatogonia in irradiated-only mice, but this was significantly increased to 6.3% after GnRH-ant treatment, and to 5.7% after GnRH-ant + flutamide treatment. However, the addition of FSH to GnRH-ant treatment resulted in differentiated donor-derived germ cells in only 3.3% of tubules. This result suggested that FSH supplementation around the time of transplantation in GnRH-suppressed mice actually reduces colonization and/or differentiation of transplanted SSCs. Further, the androgen suppression with GnRH-ant was sufficient to achieve maximal recovery as flutamide treatment did not have any further promotional effect. These results are useful in further investigations in choosing a hormone suppression regimen in combination with spermatogonial transplantation as a treatment to restore fertility in young men after cytotoxic cancer therapy.

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113

MEN MATTER: MEN WITH INFERTILITY SHOULD HAVE A COMPREHENSIVE FERTILITY INVESTIGATION

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(Presented By: Dana Livne-Segev, MD)

Introduction: Male Infertility is common,but since couples with male infertility may be treated with IUI/IVF, without any investigation for the man, often a comprehensive male factor investigation is omitted. Such an evaluation is not only important for the sake of treating the male infertility but also may detect general health issues, including life-threatening pathologies. Our objective was to describe the findings identified during a comprehensive male infertility evaluation.

Methods: In this retrospective study we extracted the data from charts of all infertile patients who had at least one abnormal semen analysis (SA) who were referred to a university affiliated male infertility referral clinic between August-November 2014. The comprehensive male fertility evaluation included an i medical history, physical examination and hormonal profile (testosterone, LH, FSH and E2) and scrotal ultrasound (US).

Results: 185 patients were included with average age, BMI and infertility duration of 36.3 years, 27.5 and 39 months, respectively. Average SA volume, count, motility and morphology were 1.5ml, 16.3 x 106/ml, 20.2% and 13%, respectively. 24 patients (13%) have azoospermia and one (0.5%) had aspermia. Overall, 172 (93%) men had at least one abnormality found during the comprehensive workup. Medical history targeted at male infertility assessment revealed abnormal findings in 67%, in which the most common was previous testicular pathology in 27% (testicular torsion, cryptorchidism,undescended testis, infections, pain, and reported varicocele). Physical examination detected pathological findings in 57% of patients in which varicocele was the most common finding 46% ,with other findings like small testicular size,abnormal testicular position, cryptorchidism,non-palpable vas deference, and testicular pain/sensitivity being less common. Abnormal hormonal profile was found in 51.4% with low testosterone being found in 36%. Scrotal US demonstrated abnormal findings in 71.2% of cases, including varicocele in 54%of patients and solid masses in 3% of investigated patients. Other abnormalities were abnormal testicular position, calcifications, testicular/epididmal cysts and hydroceles.

Conclusion: A detailed comprehensive initial evaluation leads to a very high detection rate of abnormalities. Many of these conditions if treated could result in improved fertility for the man. Some of the conditions (testis masses and hypoandrogenism) are also potential health hazards for the men.

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114

A MULTIDISCIPLINARY MODEL FOR EXPERIMENTAL TESTICULAR TISSUE BANKING TO GENERATE SPERMATOGENESIS IN THE FUTURE USING TRADITIONAL & ELECTRONIC MEDICAL RECORD ALERT SYSTEMS: DESCRIPTION OF A PROGRAM

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(Presented By: Hooman Sadri-Ardekani, MD, PhD)

Introduction: Over the past few years fertility preservation has become a prominent area of interest in reproductive medicine. In immature boys at risk of permanent infertility preserving testicular tissue containing spermatogonial stem cells (SSC) is the only option. Patient selection and referral for fertility preservation consultation before starting gonadotoxic treatment or going through a surgical procedure is the most challenging issue and often is missed. Our objectives were to to describe a successful methodology using traditional referral and electronic medical record system alerts for testicular tissue banking.

Methods: Boys 18 years and younger prior to starting gonadotoxic treatment or undergoing orchiopexy for bilateral undescended testes (B-UDT) were offered testicular tissue biopsy to store SSC under the same general anesthesia for either port-a-cath-insertion or orchiopexy. Patients were referred by the pediatric oncology, pediatric urology, or pediatric surgery services by traditional referral means. An alert system was also developed to identify potentially eligible patients using the institutional electronic medical record system (Epic, Verona, Wisconsin, USA). In addition, at least one of male reproductive medicine specialists regularly attended pediatric tumor board meetings to review and discuss potential cases for banking with other board members.

Results: In a one year period, from July 2014 to July 2015, 14 cancer and 19 B-UDT boys were eligible for testicular banking. The acceptance rates for biopsy were 78.6% and 78.9% in cancer and B-UDT boys respectively. Twenty-three boys (70%) underwent biopsy; one cancer and two B-UDT boys were excluded from biopsy based on their medical condition. The testis biopsy was performed by 7 different surgeons using a standardized protocol. The mean age of boys participating in banking was 5.6 and 5.7 years in cancer and B-UDT boys. The average sizes of biopsy specimens were 108 mm3 and 40.4 mm3, which represent approximately 5.2% and 3.3% of whole testes in cancer and B-UDT boys, respectively. The average numbers of total spermatogonial cells per seminiferous tubule were 2.3 times higher in boys with cancer than boys with B-UDT, ($p<0.001$).

Conclusion: We have established an effective, reliable and safe multidisciplinary team approach for boys at risk of infertility to bank testicular tissue. Using both traditional and electronic systems allowed us to cover most of the eligible patients in our health system.

115

HUMAN SPERMATOGONIAL CELLS STABILITY AFTER 24 HOURS TRANSPORTATION AND FREEZE-THAW

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(Presented By: Hooman Sadri-Ardekani, MD, PhD)

Introduction: Testicular tissue cryopreservation to preserve spermatogonial stem cells (SSC) for future generation of spermatogenesis is a potential option for patients at risk of permanent sterility, such as cancer survivors. Although utilizing SSC to reintroduce fertility is still experimental, trend to consider SSC banking is becoming more feasible and popular. However not all centers have ability of receiving and processing the tissue for immediate cryopreservation. It is also not well known whether a tiny harvested tissue via biopsy will be stable after transportation and freeze-thaw to isolate and propagate enough SSC for future clinical application. Our objectives were to contrast and compare various methods of testicular tissue collection and storage for future clinical applications.

Methods: Normal human testis tissue biopsy from 3 adolescent and adult individuals was collected and processed following our protocol utilized presently for pediatric cancer children, equating to biopsies of less than 10 mm3 tissue. Four different conditions were compared including (1) fresh, (2) transported (in 24 hours by using a specially designed kit), (3) frozen-fresh (immediately frozen), (4) frozen-transported. Morphology integrity by means of hematoxylin & eosin (H&E) staining and transmission electron microscopy (TEM), yield of cell recovery, apoptosis, in vitro propagation ability of SSC for at least 55-days and gene-epigenome expression level at the end of cell culture were analyzed in all conditions.

Results: Cell morphology and apoptosis as well as structure of testis tissue were compromised over the transportation and freeze-thaw process. However we were able to isolate 2-12 X 103 viable cells per mg of tissue in different conditions. The isolated cells grew in all 4 conditions for minimum of 55 days. Gene expressions of different spermatogonial markers previously used as SSC enrichment markers, including PLZF, UCHL1, CD9, GPR 125, CD49f, CD29, SSEA4 and Thy1 as wells as epigenetic markers including PEG3 and MEG3 were stable in all conditions. These results together showed our collection-transportation kit worked properly.

Conclusions: Collection, 24 hours transportation and freeze-thaw

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of tiny amount of human testicular tissue were successful in preserving testicular tissue to propagate SSC for future clinical applications. By using our collection-transportation kit, one specialized central SSC tissue bank could be a repository for many regional medical centers.

116

DIFFERENTIAL EFFECTS OF SILDENAFIL AND TADALAFIL IN HUMAN PENILE SMOOTH MUSCLE CELLS: NEW INSIGHTS FOR OLD MECHANISMS

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(Presented By: Bashir Mahmoud Rezk Atteia, PhD)

Introduction: Normal penile smooth muscle structure and function are necessary for the initiation and maintenance of erection. Improvement in the relaxation of the cavernosal smooth muscle via phosphodiesterase 5 inhibitors (PDE5i) is attributed to the inhibition of PDE5 enzyme resulting in accumulating of cGMP and reduction of cytosolic calcium. This study investigates the additional mechanism(s) of the effects of sildenafil and tadalafil on human penile smooth muscle cells.
Methods: Primary human corpora cavernosa smooth muscle cells (HCCSMC) were isolated from penile tissues. HCCSM cells (passages: 3-7) were seeded in petri dishes (1 x 106/ml) for 24 hr. Then, the cells were treated with 100 μM of sildenafil or 100 μM tadalafil for 4 h and 24 h. Results: HCCSMC showed an elevation of mRNA expression of nNOS with 100 μM of sildenafil (19.4 ± 7.4, p = 0.035) compared to tadalafil (8.5 ± 7.4, p = 0.19). However, the mRNA expression of endothelial eNOS was slightly downregulated with sildenafil (0.64 ± 0.22, p = 0.17) while, tadalafil induced insignificant increase in the mRNA expression of eNOS (2.8 ± 1.6, p = 0.19). Although both sildenafil and tadalafil are phosphodiesterase 5 inhibitors, the PDE5 mRNA expression increased after treatment with sildenafil (2.15 ± 0.53, p = 0.20) but decreased with tadalafil (0.37 ± 0.2, p = 0.12). On the other hand, levels of cGMP were significantly elevated with sildenafil (1.82 ± 0.23 pM/mg protein compared to control 1.28 ± 0.15 pM /mg protein with p value of 0.04). However, tadalafil showed no effect on the cGMP levels compared to control. PKG mRNA expression levels were increased 6.88±3.41 fold with sildenafil (p = 0.08), and 2.3 ± 1.07 fold with tadalafil (p = 0.2).
Conclusion: These findings suggest differential effects of sildenafil and tadalafil on HCCSMC. Sildenafil elevates mRNA levels of nNOS and PKG and cGMP protein levels. However, tadalafil enhances eNOS mRNA expression while, downregulating PDE5 mRNA. These need further investigation possibly using in vivo approaches.

117

CONTEMPORARY ANALYSIS OF THE SURGICAL MANAGEMENT OF MEN WITH PEYRONIE'S DISEASE WITH HOURGLASS DEFORMITY

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(Presented By: Faysal A. Yafi, MD, FRCSC)

Introduction: Less than 10% of Peyronie's disease (PD) patients have an hourglass deformity. We investigated the outcomes of two surgical interventions for PD patients with hourglass deformity —partial excision and grafting (PEG) or inflatable penile prosthesis (IPP) implantation.
Methods: Retrospective data was collected from two centers: Technical University of Munich (PEG) and Tulane University Medical Center (IPP). Collected variables included patient demographics, pre- and post-treatment sexual function, penile vascular measurements, and treatment outcomes.
Results: A total of 50 PD patients with hourglass deformity (26 PEG [group 1] and 24 IPP [group 2]) were included in this study. Patients in group 1 had higher mean pre-operative Sexual Health Inventory for Men (SHIM) scores (22.2 vs. 10.3, p<0.0001), required less ED treatment (35% vs. 79%, p=0.005), and had more non-vascular etiology on pre-operative penile duplex Doppler ultrasound (PDDU) (77% vs. 21%, p<0.0001). There were no intra-operative complications, two patients in group 1 had post-operative glans hypoesthesia, and one patient in group 2 required surgical revision. All patients in both groups had significant ≥20% improvements in penile curvature with mean changes of 68.1 degrees (12.7) in group 1 and 49.6 degrees (13.5) in group 2, <0.0001. Resolution of hourglass deformity was achieved in 85% of patients in group 1 and 100% of group 2, p=0.045. The mean post-operative change in SHIM score was -0.3 (1.3) in group 1 and 16.7 (4.7) in group 2, <0.0001.
Conclusion: Both surgical options provide excellent outcomes for well selected patients with PD and an hourglass deformity. PEG can be offered to patients with good erectile function, while the IPP remains the preferred option for patients with poor erections.

118

MIRABEGRON ELICITS SMOOTH MUSCLE RELAXATION OF HUMAN AND RAT CORPUS CAVERNOSUM TISSUES VIA NON NITRIC OXIDE-CGMP PATHWAYS

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Introduction: The β3-adrenergic receptor (AR) subtype of the sympathetic nervous system has been well characterized at the structural and molecular levels. Stimulation of β3-ARs localized on smooth muscle cells may play a physiological role in mediating penile erection. Mirabegron, a selective β3-AR agonist, has been recently developed for the treatment of

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overactive bladder, and may offer an alternative pharmacological option for the treatment of erectile dysfunction (ED). We sought to examine the effects and mechanism of action of mirabegron in rat and human erectile tissues.

Methods: Human corpus cavenosum (CC) specimens were obtained from 17 patients with ED undergoing penile prosthesis implantation. Mirabegron-elicited relaxation responses (10-8-10-3 M) on phenylephrine (Phe)-induced contraction were performed using human and rat CC strips in the organ bath. The effects of inhibitors [NG-nitro-L-arginine methyl ester (L-NAME, 100μM), 1H-[1,2,4]-oxadiazolole-[4,3-a] quinoxalin-10one (ODQ, 30μM), methylene blue (20μM), SR59230A (β3-AR blocker, 1 μM), and fasudil (Rho-kinase-ROCK inhibitor, 0.1 μM)] on mirabegron-induced relaxation responses were evaluated. After drug exposure, immunohistochemistry was employed to localize β3-AR in CC smooth muscle cells and cGMP levels were measured by enzyme-linked immunosorbent assay.

Results: Mirabegron inhibited Phe-evoked CC contractions in a dose-dependent manner and SR59230A antagonized mirabegron-induced relaxations in human and rat CC. Inhibitors of L-NAME and ODQ as well as methylene blue did not affect the mirabegron-induced relaxation responses. Mirabegron responses (between 0.1 and 10μM) were enhanced by the ROCK inhibitor, fasudil. KCl-induced contractions in human and rat CC were partially inhibited by mirabegron. Although intracavernosal mirabegron (doses of 0.1 – 1 mg/kg) had a minor effect compared to the vehicle administered alone on ICP, it decreased MAP. β3-ARs localized mostly to smooth muscle cells of human and rat CC. The inhibitory effects of mirabegron on contractile activity were independent of changes in tissue cGMP.

Conclusion: Mirabegron markedly relaxes isolated CC by activating β3-ARs independently of the NO-cGMP pathway. These results may provide support for further clinical studies using combinations of mirabegron with ROCK and phosphodiesterase-5 inhibitors for the treatment of ED.

119

LOW CALCULATED FREE TESTOSTERONE LEVELS CORRELATES SIGNIFICANTLY WITH SEXUAL DYSFUNCTION SYMPTOMS IN BORDERLINE LOW TOTAL TESTOSTERONE PATIENTS

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(Presented By: Robert S. Tan)

Currently, the treatment of hypogonadism is based on guidelines that define a lower limit of serum testosterone level. There is variation of definition of the lower limit of total testosterone (TT) ranging from 200-350 ng/dl and free testosterone (FT) from 5-10 ng/dl. Insurance companies determine eligibility of treatment based on these guidelines. Our study looks at patients who seek treatment at the Low T Centers that have borderline low TT between 350-450 ng/dl and who may not qualify for treatment.

In this retrospective study, blinded data was extracted from EMR (Advanced MD). As screening for eligibility for treatment, besides having blood work for testosterone levels, patients complete a form determining primary and secondary symptoms relating to hypogonadism. TT was determined initially using the Qualigen® competitive chemiluminescence method and confirmed, if needed, on LC/MS. FT is calculated using the Vermuelen algorithm. Patients with TT 350-450 and requiring a symptom checklist by insurance in 42 clinics across the U.S. from June-November 2015 were included. Data was entered into an Excel spreadsheet and descriptive and comparative analysis applied.

Our study group (n=593) were males, with a mean TT = 394 ng/dl (range 350-450) and mean FT= 7.0 ng/dl (range 3.3-13.7). In this group, we found a high percentage of patients with primary symptoms of loss of libido (86%) and loss of spontaneous erections (68%). Less common primary symptoms were hot flashes (11.4%), decreased testes size (4%), gynecomastia (3.8%), and hair loss (1.5%). Less specific secondary symptoms such as fatigue, mood, mental and weight changes were however very common. Of the patients that had TT between 350-450 ng/dl; 41% had FT < 6.5 ng/dl and 96% had < 10 ng/dl. 77% of patients with FT <6.5 and 76% with FT 6-5-10 complained of loss of libido as compared to 60% that had FT >10, the difference of which is statistically significant (p= 0.01).

Clinicians are often faced with therapeutic dilemmas in symptomatic but marginally hypogonadal patients based on TT level measurements. During screening, they should have not only their total testosterone levels repeated, but a FT level. We found that FT may help distinguish patients with sexual dysfunction symptoms and borderline total testosterone. Further study is needed to see if borderline hypogonadal patients treated with testosterone can have their sexual symptoms improved.

120

NEURAL ANDROGEN RECEPTOR AND MYELIN REPAIR: ANALYZES OF ANDROGENIC POTENTIAL OF M.PRURIENS IN AGED RAT DORSAL NERVE OF PENIS REPAIR

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(Presented By: Seppan Prakash, MSc, PhD)

Introduction: Degenerative change in Schwann cell and demyelination of dorsal nerve of penis (DNP) can be a predominate factor behind Erectile dysfunction (ED). The possible role of androgen receptor in Schwann cell regeneration and myelin repair of DNP is seem to be least explored area in this field. Mucuna pruriens (M.pruriens), a leguminous plant identified for its properties like aphrodisiac and improving fertility, including androgenic ability in the seed of M.pruriens has been the motivating force for this study. Our objective was to analyze the role of androgen receptors in Schwann cell in neural regeneration of DNP in ageing in relation to penile erection. To test the androgenic potential of M.pruriens in DNP repair, using Wistar albino rat as experimental animal model.

Methods: Grouping: Young (3 months), Aged (24−28 months), Aged+M.pruriens and Young+M.pruriens (ethanolic extract of the seed at dose of 200 mg/kg b.w for 60 days). Rats were subjected to hypo−thalamo−hypophysial−gonodal axis, nerve

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conduction velocity (NCV) and penile reflex. DNP processed for electron (EM) & light microscopic studies, nNOS and NaDPH diaphorase. Osmium tetroxide stained slides were used for histometric analysis. Immuno-localization of androgen receptor and in-situ demonstration of apoptotic cells by TUNEL staining.
Results: Aged rats showed significant disturbance in hypo−thalamo−hypophysial−gonodal axis, degenerative changes in DNP includes demyelination, reduced diameter and number of myelinated fibers and in EM study, vacuolization and indentation of the myelin sheath and Schwann cells degeneration were seen. nNOS and the cofactor (NaDPH diaphorase) were reduced along with low levels of antioxidants. NCV was slow in aged rats and concomitant poor penile reflex. Androgen receptors were considerably reduced in the aged rats DNP with increased apoptotic cells. These pathological changes were remarkably reduced or recovered in M.pruriens treated aged rats. No pathology was seen in Young+M.pruriens.
Conclusion: Androgen receptors in DNP i.e. Schwann cells in adult rats and which were diminish in aged rat DNP. Significant increase in androgen receptor was observed in M.pruriens treated aged rats DNP along with reduction or recovery in overall structure and function status. These observations indicative of neural androgen receptor as a promising therapeutic target for myelin repair by Schwann cells regeneration and preserving DNP integrity towards restoring erectile function.

121

SUPRAPUBIC FAT REDUCTION IN PATIENTS WITH HIDDEN PENIS

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(Presented By: Ahmad Tarek Motawi, MD, MSc, FECSM)

Introduction: Hidden penis, inconspicuous and concealed penis are synonyms for the same entity "buried penis" where the penile shaft is hidden below the surface of the prepubic fat to an extent that gives an impression of a short penis, despite corporeal length being normal. The aim of our work is the reduction of the amount of the suprapubic fat by liposuction to reveal the buried penis and assess the results, patient's satisfaction and possible complication.
Methods: The procedure of suprapubic liposuction was carried out on ten men who were attending the outpatient clinic of the Andrology department, faculty of medicine Cairo University, complaining of small sized penis. Pre, immediate (on table) post-operative and 3-months post-operative skin to tip flaccid and stretched penile length was measured. Patients' satisfaction was assessed using 5-point Likert scale.
Results: There was a statistically significant increase between the pre and postoperative flaccid and stretched penile length in our patients with p value <0.001 in both.
Conclusion: An acquired adult buried penis is a correctable problem. Simple buried penis due to excess fat at the mons pubis should be differentiated from other complex cases that may require combined techniques to achieve good results with minor complications. Suprapubic liposuction is a very safe and successful procedure with minor or no complications if performed meticulously. It could improve self-esteem along with the associated physical and aesthetic concerns.

122

HYPOGONADAL MEN WITH SEXUAL DYSFUNCTION BENEFIT FROM LPCN 1021 (ORAL TESTOSTERONE) – SOAR (STUDY OF ANDROGEN REPLACEMENT) TRIAL

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(Presented By: Mohit Khera, MD, MPH)

Introduction: Sexual dysfunction (SD) has been associated with hypogonadism. Testosterone (T) therapy is indicated for treating hypogonadism and related symptoms. Currently, most T therapy is delivered via topical formulations, which have some undesirable attributes including risk of transference and skin irritation. LPCN 1021 is a novel oral T undecanoate formulation that may avoid some undesirable attributes of non-oral T.
Methods: SOAR was a randomized, active-control, 2-arm, 52-week, open-label, multicenter, dose-titration trial in 314 hypogonadal men (T<300ng/dL on 2 separate days) 18 to 80 years old. Subjects were randomized to LPCN 1021 (n=210) or Androgel 1.62% (n=104). Medical history for 88 subjects (28%) included conditions of SD at baseline. Subjects with SD had poorer quality erections (p=0.011) and more difficulty maintaining erections (p=0.017) than subjects without SD at baseline. The LPCN 1021 dose could be titrated up or down at Weeks 4 and 8 based on 24 h PK (eg. T Cmax and T Cavg24h). Androgel was titrated based on manufacturer's instruction. Sexual function and mood changes were assessed by the Psychosexual Daily Questionnaire (PDQ) for 7 days preceding visits. Quality of life (QoL) was assessed by the SF-36 questionnaire at Weeks 1 and 52.
Results: At baseline, subjects with SD, compared to those without, were significantly older (p=0.001), had lower estradiol levels (p=0.043), but comparable T levels. Erectile function was significantly (p<0.05) worse for subjects with SD than without SD at baseline. Subjects with SD had significant improvement (p<0.05) post LPCN 1021 for SF-36 subscores of role physical, vitality, mental health and mental component summary and all PDQ subscores (sexual desire, activity, sexual pleasure with and without partner, erectile function, and positive and negative mood). Significant (p<0.05) improvement occurred in subjects with SD post LPCN 1021, but not post Androgel, for mental health, pleasure without partner, negative mood and maintained erection. Significant improvement occurred in subjects with SD

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post Androgel but not post LPCN 1021 emotional. Mean T Cavg (465 vs 473 ng/dL), DHT Cavg24h (113 vs 115 ng/dL) and estradiol Cavg24h (27 vs 29 pg/mL) were comparable for subjects with and without SD, respectively, at Week 13 (efficacy visit) post LPCN 1021 therapy.
Conclusion: Twice daily administration of LPCN 1021 for 52 weeks improved several parameters of sexual dysfunction in hypogonadal men with SD.

123

SOCIOECONOMIC FACTORS ASSOCIATED WITH PENILE PROSTHESIS RECEIPT FOR ERECTILE DYSFUNCTION IN NEW YORK STATE: ANALYSIS OF THE STATEWIDE PLANNING AND RESEARCH COOPERATIVE SYSTEM DATABASE

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(Presented By: Ifeanyi Cliff Onyeji, BA)

Introduction: Socioeconomic factors could influence the decision to treat erectile dysfunction (ED) with a penile prosthesis (PP) by impacting affordability, access to specialists, ED severity, and surgical risk. Our goal was to evaluate the role of socioeconomic status (SES) on PP receipt among men with ED at the population level.
Methods: The New York State Statewide Planning and Research Cooperative System (SPARCS) database was queried from 2011–2014 to identify men diagnosed with ED in the outpatient setting without a PP, and men with a PP. SES data at the zip code level came from the 2006-2010 American Community Survey. Median household income, unemployment rate and percent below poverty comprised the SES quintile index. Differences in demographic and socioeconomic factors between the two groups were analyzed with t-test. Multivariable logistic regression was used to identify predictors of PP receipt.
Results: 60,230 men with ED had no history of PP surgery and 2,876 men had received a PP for ED. The PP group was older (62.6 ± 9.9 vs. 58.2 ± 12.4 years, p<0.001). Compared with 5% of whites, 3.9% of blacks (p<0.001), and 2.6% of Asians (p<0.010) received a PP. Metropolitan residents had higher PP rates than their rural counterparts (4.75 vs. 0.723%, p<0.001), as did men with lower comorbidity scores vs. those with higher scores (4.76% vs. 1.95%, p<0.001). Men in the highest SES quintile also had higher PP rates relative to the lowest quintile (6.3% vs. 2.9%, p<0.001). Compared with 4.5% of privately insured patients, 0.05% of Medicare (p<0.001) and 0.7% Medicaid patients (p<0.001) had received a PP. On multivariable analysis, older age (OR 1.034, 95% CI: 1.030-1.038) was significantly associated with PP receipt, whereas greater comorbidity severity (OR 0.331, 95% CI: 0.124 – 0.514), rural residency (OR 0.106, 95% CI: 0.047-0.238), low SES (OR 0.565, 95% CI: 0.488 – 0.654) and Medicaid insurance (OR 0.205, 95% CI: 1.053 – 1.369) were significantly associated with not receiving a PP.
Conclusion: Despite the coverage of PPI for ED by government insurance, low SES, residency in non-metropolitan areas and Medicaid insurance are still associated with reduced access to PP surgery for ED.

Multivariable analysis of predictors of PP receipt

Variable	Adjusted OR (95% CI)	p-value
Age	1.034 (1.030 - 1.038)	<0.001
Urbanity		
Metro	REF	--
Urban	0.230 (0.170 - 0.312)	<0.001
Rural	0.106 (0.047 - 0.238)	<0.001
Comorbidity Index		
Category 1	REF	--
Category 2	0.636 (0.568 - 0.712)	<0.001
Category 3	0.331 (0.124 - 0.514)	<0.001
SES_Index		
Quntile 1	0.565 (0.488 - 0.654)	<0.001
Quntile 2	0.876 (0.767 - 1.002)	0.054
Quntile 3	REF	--
Quntile 4	1.012 (0.890 - 1.149)	0.86
Quntile 5	1.200 (1.053 - 1.369)	0.006
Insurance Type		
Private	REF	--
Medicare	1.003 (0.911 - 1.105)	0.948
Medicaid	0.205 (0.147 - 0.286)	<0.001

124

EFFECTS OF MICRONUTRIENT SUPPLEMENTATION ON FEMALE SEXUAL FUNCTION: A RANDOMIZED TRIAL

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(Presented By: Nikolaos Sofikitis, Professor)

Introduction: Assisted reproductive technology is an expensive and with potential risks mode of alleviation of infertility. Thus, healthy female partners of men with subnormal values of the standard parameters of semen analysis often choose to attempt to achieve pregnancies via sexual intercourses. We evaluated the role of Female-PROFERTIL (F-PRO; Lenus Pharma, Vienna, Austria) on the sexual function of female partners of infertile men.
Methods: F-PRO (2 caps/day; group A) or L-carnitine (1000 mg/day; group B; positive controls) were administered to 18 and 17 female partners, respectively, of men with values of sperm motility within the normal range (but near the lower limits) and normal values of sperm concentration and sperm morphology for 12 weeks. Another group of 16 female partners (group C) of men with same characteristics for semen analysis parameters (as the male partners of women of groups A and B) served as a negative control group and received no pharmacological treatment. The experimental period in group C was considered to be 12 weeks. The present study was initially scheduled to include 51 infertile couples with the above characteristics. When each female partner was recruited she was randomly assigned to one of the three groups. Damiana, selenium, vitamin E, ignama, folic acid, and fatty acids-omega-3 are some of the components of F-PRO. The

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outcome of the Female Sexual Function Index (FSFI; Marital Therapy 26:291, 1998) was evaluated before administering the pharmaceutical agent in each of groups A and B, and after the end of the treatment. The same variable was assessed in group C before and after the end of the experimental period.

Results: Within group A, the mean value of FSFI was significantly smaller before treatment than afterward (16±7 vs 26±5). Wilcoxon test for paired observations was used for statistical analysis. A probability P smaller than 0.05 was considered to be statistically significant. By contrast, within group B and C, the difference in the mean FSFI before and after the experimental period was not significant.

Conclusion: F-PRO significantly increases the FSFI. This might be attributable to the action of ignama which has been suggested to increase the peripheral serum concentration of estrogens.

125

COMPARISON OF SPERM CHROMOSOMAL ABNORMALITIES EVALUATED IN ONE TEENAGER AND FOUR ADULTS WITH KLINEFELTER SYNDROME

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(Presented By: Rives Aurelie)

Introduction: Klinefelter syndrome (SK) diagnosis is generally performed in adulthood in infertile males and exceptionally in childhood or adolescence. Spermatogenesis alterations observed in KS is related to germ cell depletion that intensifies at puberty and beyond. Fertility preservation should be discussed in teenager KS, to optimize the chance to retrieve spermatozoa in ejaculate or after testicular biopsy. Spermatogenesis initiation is physiologically chaotic and the issue of sperm quality produced at this time is questioned in KS. The aim of our study was to assess the rate of chromosomal abnormalities in sperm of one KS teenager compared to four KS adults.

Methods: The study interested one KS teenager (46,XY/47,XXY) whose diagnosis was performed during prenatal karyotyping for maternal age and four KS adults with primary infertility (two 47,XXY males and two 46,XY/47,XXY males). The teenager performed a first semen sampling at 15 years early after puberty and a second one at 18 years. Spermatozoa were present in the ejaculate of the teenager and the four infertile men. The rate of chromosomal abnormalities was evaluated by fluorescence in situ hybridization for X, Y and 18 chromosomes. The primary endpoint was the comparison of sperm aneuploidy in semen samples of the KS teenager at the two different ages versus the four KS adults.

Results: Sperm parameters of the KS teenager remained stable during the study period, with hypospermia and severe oligozoospermia oscillating between 0.1 and 0.5 ml/l. The first sperm sampling of the teenager showed a rate of aneuploidy comparable to that of KS adults (homogeneous or mosaic kayotype) and a lower diploid rate ($p < 0.0001$). The second sperm sampling showed lower rates of aneuploidy ($p<0.0001$) and diploidy ($p<0.0001$) compared to those of KS adults (homogeneous or mosaic karyotypes). The aneuploidy rate was higher in the sample peformed at 15 years compared to that achieved at 18 years (0.25 % vs. 0.93 %, $p < 0.0001$), the diploidy rate was comparable.

Conclusion: Our results suggest a qualitative deterioration of spermatogenesis over time in KS patients. Sperm aneuploidy being greater in early post-puberty, fertility preservation should be performed after spermatogenesis stabilization. Nevertheless, the study of a larger cohort of adolescents with KS is necessary confirm the results of our study.

126

AWARENESS ABOUT FERTILITY OPTIONS FOR THE TRANSGENDER COMMUNITY

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Introduction: Figures from United States suggest that 0.3-0.5%of people identify as transgendered. Limited data suggest that many transgendered people have not considered fertility preservation; even though, hormonal and surgical transitioning is incompatible with fertility. Guidelines of World Professional Association for Transgender Health recommend that transgender patients should be counselled on the option of fertility preservation prior to hormonal interventions. How many patients are aware or who have availed themselves of this option remains unclear.

Methods: A prospective anonymous questionnaire study to identify patient demographics, characteristics, planned knowledge about fertility options and fertility therapies, was conducted in two universities in Toronto with special expertise in the treatment of the trans gender population.

Results: Data on 22 patients who completed the survey was analyzed. The average age was 24. A total of 68% of the patients have begun hormone therapy with 85% of the remaining 32% planning on starting hormone therapy in the future. None of the patients had begun surgical transitioning yet, but 36% are planning surgical transitioning in the future. Two of 22 (9%) have children. Of the total group, only one indicated a clear desire to have children in the future, while 11/22 were unsure if they wanted children and 10/22 were certain that they did not want children in the future. 41% thought that the option to have children was somewhat or very important for the sense of self. While 100% felt the option to bank gametes prior to hormone therapy was important, only 40% reported that a discussion about fertility preservation had occurred with their physicians and only 23% were willing to consider stopping therapy to bank gametes. Leading barriers preventing from patients seeking egg/sperm banking were cost and treatment delay, and the actual concept/process they have to go through was a barrier.

Conclusion: This pilot study indicates that trans patients need more detailed information regarding their fertility options, especially because some of the changes from transitioning are irreversible. A lot of factors contribute to their fertility decision,

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such as their personal background and emotional and economical support, and their knowledge regarding fertility options. It seems that on one hand, all patients want the opportunity to preserve fertility option, but on the other hand most of them do not avail and do not plan to avail themselves of this option.

127

SIMVASTATIN INDUCES CELL CYCLE ARREST AND INHIBITES PROLIFERATION OF BLADDER CANCER CELLS VIA PPAR AND ERBB SIGNALING PATHWAYS

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(Presented By: Gang Wang, MSC)

Introduction: Statins are currently one of the most common drugs for old patients with hyperlipidemia, hypercholesterolemia and atherosclerotic disease, by reducing cholesterol and anti-lipid properties. Statins have also be reported an anti-tumor effect on bladder cancer (BCa), but the underlying mechanism is still unknown. Microarray analysis using human BCa and normal bladder tissues, suggested human BCa might be connected with fatty acid and lipid metabolism via Peroxisome Proliferator-Activated Receptors (PPARs) and ErbB signaling pathways. We aim to investigate the effect of simvastatin, a highly used statin, on proliferation and metastasis in distinct BCa cells.
Methods: Effects of Simvaststin on cell on proliferation and metastasis were investigated in BCa cells EJ, 5637 and T24. Simvastatin treatment could trigger alterations of cell growth and proliferation, analyzed by MTT and clonogenic survival assay. Cell cycle arrest and apoptosis were analyzed by flow cytometer, as well as proteins involved in cell cycle and epithelial-mesenchymal transition (EMT) were analyzed by Western blot. Immunofluorescence stainings were used to investigate alterations of E-cadherin, N-cadherin and Vimentin by simvastatin treatment.
Results: Simvastatin could reduce cell proliferation in a dose- and time-dependent manner, accompanied by upregulation of PPAR alpha/gamma and downregulation of ErbB1/2. Furthermore, flow cytometer analysis showed simvastatin could induce cell cycle arrest at G1/G0 phase, demonstrated by downregulation of cyclin D1 and CDK4/6. Moreover, simvastatin suppressed bladder cancer cell metastasis through inhibiting cancer cells to undergo EMT by inducing of E-cadherin and reducing of N-cadherin, Vimentin, etc. Importantly, cell cycle arrest and decreasing of ErbB1/2 caused by simvastatin could be recovered by PPAR antagonists.
Conclusion: Simvastatin could inhibit BCa cell proliferation and induce G1/G0 phase cell cycle arrest via PPARs, ErbB, EMT and cyclin D1 - CDK4/6 pathways, and therefore simvastatin could be a potential agent for preventing bladder cancer.
Funding: Wuhan Clinical Cancer Research Centre for Urology and Male Reproduction (303-230100055), National Natural Science Foundation of China (81172434) and Fundamental Research Funds for the Central Universities (2042015kf0081).

128

NOVEL STRATEGY FOR PURIFICATION OF PROSTATE SPECIFIC ANTIGEN (PSA) FROM HUMAN SEMINAL PLASMA

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Introduction: PSA is a chymotrypsin-like serine protease secreted by the prostate and incorporated into the seminal plasma during ejaculation. Although PSA is predominantly known as a prostate cancer biomarker, its normal physiological function is the enzymatic cleavage of the semen coagulum following ejaculation. The proteolytic substrates of PSA in semen include the seminogelins and galectin-3. Furthermore, a limited number of studies have indicated a functional role(s) for PSA in the pathobiology of prostate cancer. Methods previously used to purify PSA include ion exchange, thiophilic gel affinity, and immunoaffinity chromatorgraphy. Significantly, multiple strategies reported for PSA purification either utilize conditions that could chemically-modify PSA or have not been adequately validated for producing sufficiently purified PSA. The objective was to develop an effective and efficient protocol for purification of non-modified, enzymatically-active PSA from human seminal plasma.
Methods: Lectin blot analysis was utilized to assess chemical modification of the PSA glycan structure. PSA was isolated from seminal plasma by continuous elution electrophoresis on a Bio-Rad Prep Cell Model 491. PSA enrichment was monitored by SDS-PAGE and total protein staining. Purity and yield of the final PSA product was assessed by ELISA, Western blot, total protein stain, and protein sequencing by mass spectroscopy. PSA enzymatic activity was evaluated by proteolytic cleavage of galectin-3.
Results: Lectin blotting demonstrated that incubation with low pH buffer altered the carbohydrate structure on the PSA glycoprotein. Two round of continuous elution electrophoresis yielded enzymatically-active PSA at greater than 98% purity. PSA purified by this method exhibited a higher degree of purity and enzymatic activity than a commercially-available PSA preparation.
Conclusion: Continuous elution electrophoresis of seminal plasma yielded highly-purified, enzymatically-active PSA. This method avoided low pH conditions that would alter the PSA glycan structure and provided a protein yield higher than what would be expected for immunoaffinity chromatography. We anticipate that this novel purification strategy will provide adequate supplies of non-chemically-modified, enzymatically active PSA for the investigation of PSA in normal reproduction and prostate cancer, and for the development of improved PSA-based screening tests for prostate cancer.

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130

DEPLETION OF THIOLS AND INCREASED 4-HYDROXYNONENAL DURING CRYOPRESERVATION OF STALLION SPERMATOZOA LEAD TO CASPASE ACTIVATION AND CELL DEATH

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(Presented By: Fernando Juan Pena Vega, PhD)

Oxidative stress has been linked to sperm death and the accelerated senescence of cryopreserved spermatozoa. However, the molecular mechanisms behind this phenomenon remain poorly understood. Reactive oxygen species (ROS) are considered relevant signaling molecules for sperm function, only becoming detrimental when ROS homeostasis is lost. We hereby hypothesize that a major component of the alteration of ROS homeostasis in cryopreserved spermatozoa is the exhaustion of intrinsic antioxidant defence mechanisms. To test this hypothesis, semen from seven stallions was frozen using a standard technique. The parameters of sperm quality (motility velocity, membrane integrity) and markers of sperm senescence (caspase 3, 4-hydroxynonenal, mitochondrial membrane potential) were assessed before and after cryopreservation. Moreover, changes in the intracellular thiol content were also monitored. Cryopreservation caused significant increases in senescence markers as well as a dramatic depletion of intracellular thiols to less than half of the initial values ($p<0.001$) post-thaw. Interestingly, very high and positive correlations were observed among thiol levels with sperm functionality post-thaw: total motility ($r= 0.931$ $p< 0.001$), progressive motility ($r=0.904$ $p<0.001$) and the percentage of live spermatozoa without active caspase 3 ($r= 0.996$ $p< 0.001$). In contrast, negative correlations were detected between active caspase 3 and thiol contents both in living ($r= -0.896$) and dead spermatozoa ($r=-0.940$); additionally, 4-hydroxynonenal levels were negatively correlated with thiol levels ($r= -0.856$). In conclusion, sperm functionality post-thaw correlates with the maintenance of adequate levels of intracellular thiols. The accelerated senescence of thawed spermatozoa is related to oxidative and electrophilic stress induced by increased production of 4-hydroxynoneal in thawed samples once intracellular thiols are depleted.

131

EFFECTS OF PDE5 INHIBITORS ON EPIDIDYMAL RAT SPERM MOTILITY

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(Presented By: Fotios Dimitriadis, PhD)

Introduction: We evaluated the role of sildenafil, tadalafil, and avanafil on the ability of frozen rat epididymal spermatozoa to develop sperm motility post-thawing.
Materials and Methods: Epididymal caudal spermatozoa recovered from ten 10-week-old Wistar rats were washed in MTC medium and were then frozen. Six to 21 months later four aliquots A, B, C and D containing frozen/thawed washed spermatozoa were prepared from each rat. Spermatozoa contained in aliquots A, B, and C were further washed in MTC medium (Int J Androl. 2009;32(6):675-86). Then aliquots A, B, C, or D of each rat were mixed with a vehicle volume of MTC medium, or sildenafil (to a final concentration of 1 mg/ml), or tadalafil (to a final concentration of 1 mg/ml), or avanafil (to a final concentration of 1 mg/ml), respectively. Then all aliquots A, B, C, or D were incubated under 5% carbon dioxide, under 5% oxygen at 37oC for 4 hours.
Results: At the end of the incubation period, the mean value of the percentage of motile spermatozoa was significantly larger (P smaller than 0.05; analysis of variance plus Duncan test) in aliquots B (37±15) and D (47±16) compared with aliquots A (16±7) and C (13±6).
Conclusions: PDE5 inhibitors allow washed frozen/thawed rat epididymal caudal spermatozoa to obtain motility. The superior results of the avanafil and sildenafil compared with the results of tadalafil may be attributed to the suggested inhibition of PDE11 by tadalafil (Int J Impot Res. 2005;17:216).

132

MEASUREMENT OF OXIDATION-REDUCTION POTENTIAL (ORP) AS A NEWER TOOL INDICATIVE OF OXIDATIVE STRESS IN INFERTILE MEN WITH LEUKOCYTOSPERMIA

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(Presented By: Suresh C. Sikka, PhD, HCLD, CC(ABB))

Introduction: Oxidation-reduction potential (ORP) is a newer integrated measure of the balance between total oxidants (ROS) and reductants (antioxidants) that reflects oxidative stress status (OSS) in a biological system. However, the current methods to measure OSS are time consuming, expensive, have many limitations, and thus are not routinely performed. Seminal oxidative stress, especially due to presence of high number of active leukocytes (WBC) in semen (i.e., leukocytospermia) is known to affect sperm parameters and fertility. This preliminary study measures both static-ORP (sORP as mVolt) and capacity-ORP (cORP as µC) in fresh semen samples using the new electrochemical system, MiOXSYS™.
Methods: Semen samples from fifteen infertile men presenting to andrology clinic were collected after informed consent and analyzed for all important sperm parameters (including WBC content) as per WHO (2010) guidelines. A 30µl aliquot was placed on the special sensor in the calibrated MiOXSYS analyzer to measure sORP and cORP. All values were normalized to sperm concentration and expressed as Mean + SEM units/million sperm/mL.
Results: Semen samples (n=10) that showed high number of WBC (average 3.8 million/mL) termed leukocytospermia (LCS),

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showed higher sORP values (6.4 + 1.9 mVolt/million sperm/mL) compared to 1.6 + 0.3 mVolt/million sperm/mL in samples from non-LCS men (n=5) with an average of 0.2 million/mL WBC. However, cORP values (µC/million sperm/mL) were lower (5.7 + 2.9) in LCS samples and much higher (19.5 + 17.8) in non-LCS samples. A 14-fold increase in the sORP/cORP ratio was observed in LCS semen samples compared to non-LCS samples.
Conclusion: These preliminary findings using the MiOXSYS System clearly suggest that sORP reflects higher oxidation-reduction potential and is an easy to determine one-step indicator of increased oxidative stress in semen samples of infertile men especially with leukocytospermia. This newer ORP parameter as measured by MiOXSYS could potentially serve as an important additional indicator in the workup of male factor infertility. It will be important to carry out much larger standardized studies, hopefully in a multicenter format to establish such role of MiOXSYS evaluation in routine clinical practice.

134

OXIDATIVE STRESS-INDUCED SPERM DNA FRAGMENTATION AND ITS ASSOCIATION WITH VARICOCELE AND SEMEN PROCESSING

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(Presented By: Paula Intasqui Lopes, MSc, BSc)

Introduction: Sperm DNA fragmentation is one of the main mechanisms underlying varicocele-related male infertility, and it has been mostly associated with oxidative stress (OS). It has been suggested that semen processing reduces sperm DNA fragmentation, but the effect of processing on OS-induced sperm DNA fragmentation is unknown. Thus, our aim was to verify if men with varicocele present increased sperm DNA oxidative damage, if processing selects sperm with lower sperm DNA fragmentation caused by OS, and if there is an interaction effect.
Methods: Forty normozoospermic men were divided into control (n=20) and varicocele (n=20) groups. Processing was performed by discontinuous density gradient centrifugation. Sperm DNA fragmentation was assessed before and after processing by a Comet assay, in alkaline (analysis of single- and double-stranded DNA fragmentation), neutral (double-stranded DNA fragmentation), and alkaline associated with DNA glycosylase formamidopyrimidine enzyme (DNA damage by OS). Sperm were classified as Class I – intact sperm DNA to IV – high sperm DNA fragmentation. To evaluate the effect of varicocele on sperm DNA fragmentation, groups were compared before semen processing. For the study of the effect of processing on sperm DNA fragmentation, data pre- and post-processing were separately compared for the control and varicocele groups. Statistical analysis was performed using Student's t test, Mann-Whitney test, Wilcoxon test and General Linear Model. Significance was set at 5%.
Results: The varicocele group presented decreased right testicular volume, sperm morphology and total sperm count. Sperm DNA fragmentation results are presented in table 1.

Conclusion: Patients with varicocele show an increase in sperm DNA fragmentation levels, especially by the action of OS. Semen processing promotes the selection of sperm with better DNA quality, both in control and varicocele groups. The positive effects of seminal processing in the selection of sperm with intact DNA are more pronounced in patients with varicocele.

Table I: Sperm DNA fragmentation rates in control and varicocele groups, evaluated by the Comet Assay, both before and after seminal processing.

DNA Fragmentation		Control Group (n=20)		Varicocele Group (n=20)		p			
		Pre Percoll	Post Percoll	Pre Percoll	Post Percoll	Effect of the group[a] Cpre x Vpre	Effect of seminal processing[b] Cpre x Cpost	Vpre x Vpost	Interaction effect[c] Cpre x Cpost x Vpre x Vpost
Single- and double-stranded	I	48.3; 9.91	51.7; 8.67	33.1; 8.68	41.9; 10.45	0.986	<0.001*	<0.001*	0.001*
	II	41.3; 10.60	40.7; 6.73	56.1; 6.63	49.3; 7.95	<0.001*	1	<0.001*	0.008*
	III	8.9; 4.07	5.9; 3.01	6.9; 4.56	6.2; 4.72	0.170	0.024*	0.472	0.234
	IV	2.5; 2.16	1.6; 1.60	3.8; 2.37	2.5; 2.26	0.054	0.103	0.046*	0.550
Double-stranded	I	57.3; 12.86	59.7; 13.96	35.3; 7.99	41.5; 9.04	<0.001*	0.015*	<0.001*	0.008*
	II	34.8; 13.07	35.6; 12.50	57.9; 7.00	53.3; 7.39	0.034*	0.525	<0.001*	0.040*
	III	4.6; 2.39	3.4; 2.85	5.2; 3.46	4.9; 2.64	0.945	0.024*	0.658	0.298
	IV	0.6; 0.68	0.6; 1.39	1.3; 1.27	0.6; 1.23	0.035*	0.745	0.013*	0.134
Caused by Oxidative stress	I	46.7; 9.22	49.8; 12.09	30.5; 8.95	37.7; 11.51	0.851	0.042*	0.001*	0.07
	II	43.8; 10.19	41.0; 11.04	55.3; 8.30	50.0; 9.68	0.607	0.017*	0.005*	0.185
	III	6.7; 2.61	6.5; 3.43	9.4; 5.77	8.4; 4.87	0.171	0.817	0.425	0.585
	IV	2.7; 1.52	2.6; 2.09	4.7; 2.97	3.3; 2.52	0.011*	0.886	0.017*	0.070

Values expressed as: mean; standard deviation (SD)
* p <0.05, statistically significant.
a: unpaired Student's t test or Mann-Whitney test
b: paired Student's t test or Wilcoxon test
c: General Linear Model

135

PEROXIREDOXINS DIFFERENTIALLY REGULATE PHOSPHORYLATION OF PROTEIN KINASE A SUBSTRATES AND OF TYROSINE RESIDUES DURING HUMAN SPERM CAPACITATION

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(Presented By: Cristian O'Flaherty, PhD, DVM)

Sperm capacitation is an oxidative process that requires low and controlled amounts of reactive oxygen species (ROS) that trigger its associated phosphorylation events. PRDXs are antioxidant enzymes that not only act as scavengers but also control ROS action in somatic cells. Spermatozoa from infertile men have lower levels of PRDXs (particularly of PRDX6) which are thiol-oxidized and therefore inactive. Given the importance of ROS during sperm capacitation and the lack of information on how the redox signaling is regulated, we aim to determine the role of peroxiredoxins (PRDXs) during capacitation as regulators of redox signaling. Sperm from healthy donors were capacitated in the absence or presence of thiostreprton (TSP), inhibitor of 2-Cys PRDXs or MJ33, inhibitor of phospholipase A2 (PLA2) activity of PRDX6, added at different times of incubation. Capacitation was also induced by dibutyryl cAMP (dbcAMP)+3-isobutyl-1-methylxanthine (IBMX). Capacitation was determined by the ability of spermatozoa to undergo acrosome reaction triggered by lysophosphatydilcholine. Percentages of acrosome reaction were obtained using the FITC-conjugated Pisum sativum agglutinin assay. Phosphorylation of tyrosine residues and of PKA substrates were determined by SDS-PAGE immunoblotting with specific antibodies. TSP and MJ33 prevented sperm capacitation without altering sperm viability. PKA substrates and tyrosine phosphorylations were prevented in a differential fashion depending on the type and the time of addition of the inhibitor used. TSP, but not MJ33, prevented tyrosine phosphorylation

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when added after 30-120min of initiated capacitation induced by dbcAMP-IBMX. TSP promoted a greater toxic effect of hydrogen peroxide on sperm viability that was abolished by D-penicillamine (PEN) suggesting the production of an oxidative stress by inhibition of 2-Cys PRDXs. MJ33 also impaired sperm viability but its effect was not prevented by PEN, suggesting that not only peroxidase by also PLA2 activity of PRDX6 is necessary to guarantee sperm viability. In conclusion, 2-Cys PRDXs and PRDX6 PLA2 activity are necessary to allow spermatozoa to achieve fertilizing ability. PRDXs are important to allow phosphorylation of PKA substrates and of tyrosine residues associated with capacitation. PRDXs maintain low levels of ROS for capacitation, thus avoiding the toxic effects of oxidative stress that would occur if these enzymes are inactivated.

136

REGULATION OF PROTEIN PHOSPHATASE 2A DURING MOUSE SPERM CAPACITATION

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(Presented By: Maria Gracia Gervasi, PhD)

Introduction: After ejaculation mammalian sperm acquire the ability to fertilize an egg during their transit through the female reproductive tract. The changes that occur on the sperm in that period are collectively named capacitation, and they can be mimicked in vitro in a defined media. At a molecular level, this process is related to the cAMP-dependent activation of protein kinase A (PKA) and a consequent increase in tyrosine phosphorylation (pY). Our laboratory has previously shown that a downregulation of serine/threonine phosphatases is required in parallel to PKA activation for the increase in pY related to capacitation. We hypothesized that during sperm capacitation Src Family Kinases (SFKs) are activated and phosphorylate specific phosphatases that are inhibiting the downstream effectors of PKA. The present work was aimed to identify the protein phosphatase involved in this signaling pathway and to evaluate its regulation during sperm capacitation.
Methods: In vitro sperm capacitation, western blot, immunofluorescence, and affinity purification tandem to mass spectrometry were performed.
Results: Protein phosphatases PP1, PP2A, and calcineurin were found expressed in sperm and, based on experiments with pharmacological inhibitors, PP2A is our candidate for regulation during sperm capacitation. The activity of the PP2A holoenzyme can be regulated by phosphorylation or methylation of the catalytic subunit, therefore the phosphorylation and methylation status of this subunit during sperm capacitation was evaluated by western blot. Our results show that PP2A is phosphorylated during sperm capacitation and this phosphorylation is SFKs-dependent. PP2A is also methylated during capacitation which could result in changes of the regulatory subunit conforming the holoenzyme. To evaluate this, we used a custom-made affinity purification method coupled with tandem mass spectrometry analysis. Our results suggest a phosphatase interaction with members of the testis specific serine/threonine kinase family.
Conclusion: Altogether, our data support the hypothesis of a pathway parallel to PKA that regulates sperm capacitation and bring new insight into the participation of phosphatases in this process.

137

RELIABILITY ESTIMATES FOR A TEST OF SPERM CAPACITATION, A FUNCTIONAL COMPLEMENT TO SEMEN ANALYSIS

G. C. Ostermeier, PhD[1]; C. Cardona, PhD[1]; T. T. Smith, PhD[2]; M. A. Moody, MS[1]; A. J. Simpson, BS[1] and A. J. Travis, VMD/PhD[3],[1]
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(Presented By: G. Charles Ostermeier, II, PhD)

Introduction: Evaluations of semen quality are subjective. This can add unwanted variability into fertility assessments. To augment traditional semen evaluation, samples were evaluated for their ability to capacitate and become fertilization competent using localization patterns of the ganglioside GM1, the basis of the laboratory-developed Cap-ScoreTM Sperm Function Test. Here, Cap-Score precision and its variation when determined by the same and different operators were assessed.
Methods: Following liquefaction of semen samples from consenting men, sperm were washed, incubated, fixed and then evaluated via fluorescence microscopy for GM1 localization patterns. Student's t-Test employing unequal variance was done using Microsoft Excel (2013).
Results: Precision was evaluated by comparing the percent change about Cap-Score values (%Δ=(y2-y1)/y2) when 50, 100, 150 and 200 sperm were evaluated. Changes in values of 11, 6 and 5% were observed for each addition of 50 sperm ($n \geq 23$). This supports the view that Cap-Score precision was only modestly improved by counting more than 100 sperm. To be conservative, Cap-Score was determined by counting the GM1 localization patterns of at least 150 cells. To assess variation within and between readers, 8 large image files containing up to 5,000 sperm were generated. Two different readers were trained and determined Cap-Scores by randomly resampling the image 20 times, counting 150 cells each time. When scoring the same sample, individual readers reported an average SD of 3 Cap-Score units. The difference between readers when scoring the same sample ranged from 0.00 to 1.52, with an average difference of 1 between the readers for any given sample. Applying the Bonferroni correction, no difference between readers was observed for any image file (p-values ranged from 0.02 to 0.99). These data demonstrate that the same and independent readers can replicate Cap-Score values when repeatedly evaluating the same semen donor.
Conclusion: Common measures of semen quality are subjective and can vary within and among readers, making the assessment of male fertility challenging. The Cap-Score sperm Function test evaluates the ability of sperm to capacitate, a necessity for male fertility. The data presented here show that the Cap-Score Sperm Function Test is highly reproducible and reliable within and between readers, which are key considerations when attempting to diagnose male infertility.
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138

NORMAL EJACULATES, AS DEFINED BY WHO, CAN HAVE ABNORMAL CAPACITATION ABILITY.

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(Presented By: G. Charles Ostermeier, II, PhD)

Introduction: The diagnosis of male infertility is based predominantly on the results of standard semen analysis. However, standard semen analysis provides little information on sperm functional competence. Localization of the ganglioside GM1 (Cap-ScoreTM) identifies sub-populations of sperm capable of undergoing the functional maturation process known as capacitation and tracks strongly with fertility (Paniza et al., ASRM 2014). Here Cap-Score results were compared to standard semen analysis parameters obtained from men questioning their fertility.
Methods: Semen samples from consenting patients were liquefied, washed and incubated under both non-capacitating and capacitating conditions. Semen analysis was performed according to WHO guidelines. Cap-Score values were obtained via fluorescence microscopy. Statistical analyses were done using Microsoft Excel (2013) and XLSTAT (2015).
Results: Samples from 84 men referred to an infertility specialist were analyzed and had Cap-Scores ranging from 13 to 52% (mean from a population with known fertility was 39, ±7% (n=41)). An analysis of variance was done to compare Cap-Scores and sperm morphology. Samples were classified as having 0, 1, 2, 3, or ≥ 4% normal forms (scores ≥ 4% are considered normal, WHO) and mean Cap-Scores were compared among the groups. No relationship between Cap-Score and morphology was observed (P=0.67). Next, sperm concentration (range 3x106 to 210x106/mL) was compared to Cap-Score using the Pearson product-moment correlation coefficient and no connection was found (r=0.01, P=0.90). Lastly, Cap-Score was compared to total % motility (range 15 to 80%) and the two measures were determined to be independent (r=0.14 P=0.21). Multiple donors who were classified as normal by WHO criteria had Cap-Scores more than 2 SD below the normal mean, supporting the view that even normal appearing sperm can have functional abnormalities.
Conclusion: Traditional semen analysis identifies only 50% of male infertility cases. This study shows that there is little relationship between Cap-Score™ and standard semen analysis parameters. Since capacitation is necessary for fertilization, the addition of Cap-Score to traditional semen evaluations could both identify cases of idiopathic infertility and help clinicians counsel couples towards the most appropriate treatment.
Funding: Androvia LifeSciences.

139

IDENTIFICATION OF BOVINE SPERM ACROSOMAL PROTEINS THAT INTERACT WITH A 32KDA ACROSOMAL MATRIX PROTEIN

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(Presented By: Subir Nagdas, PhD)

Mammalian fertilization is accomplished by the interaction between sperm and egg. Previously, we have identified a stable acrosomal matrix assembly from the bovine sperm acrosome termed the outer acrosomal membrane-matrix complex (OMC). This stable matrix assembly exhibits precise binding activity for acrosin and N-acetylglucosaminidase. A highly purified OMC fraction is comprised of three major (54, 50, and 45kDa) and several minor (38-19kDa) polypeptides. The set of minor polypeptides (38-19kDa) termed "OMCrpf polypeptides" is selectively solubilized by high-pH extraction (pH 10.5) while the three major polypeptides (55, 50 and 45kDa) remain insoluble. Proteomic identification of the OMC32 polypeptide (32kDa polypeptide isolated from high-pH soluble fraction of OMC) yielded two peptides that matched the NCBI database sequence of acrosin-binding protein. Anti-OMC32 recognized an antigenically related family of polypeptides (OMCrpf polypeptides) in the 38-19kDa range with isoelectric points ranging between 4.0 and 5.1. Other than glycohydrolases, OMC32 may also be complexed to other acrosomal proteins. The present study was undertaken to identify and localize the OMC32 binding polypeptides and to elucidate the potential role of the acrosomal protein complex in sperm function. OMC32 affinity chromatography of a detergent soluble fraction of bovine cauda sperm acrosome followed by mass spectrometry-based identification of bound proteins identified acrosin, lactadherin, SPACA3, and IZUMO1. Co-immunoprecipitation analysis also demonstrated the interaction of OMC32 with acrosin, lactadherin, SPACA3, and IZUMO1. Our immunofluorescence studies revealed the presence of SPACA3 and lactadherin over the apical segment; whereas, IZUMO1 is localized over the equatorial segment of Triton X-100 permeabilized cauda sperm. Immunoblot analysis showed that a significant portion of SPACA3 was released after the lysophosphatidyl choline (LPC)-induced acrosome reaction; whereas, the IZUMO1 and lactadherin polypeptides remain associated to the particulate fraction. Almost entire population of bovine sperm IZUMO1 relocates to the equatorial segment during the LPC-induced acrosome reaction. We propose that the interaction of OMC32 matrix polypeptide with detergent soluble acrosomal proteins regulates the release of hydrolases/other acrosomal protein(s) during the acrosome reaction.

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140

SPERM FROM FERTILE MEN AND THOSE SEEKING FERTILITY EXAMS DIFFER IN THEIR ABILITY TO CAPACITATE

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(Presented By: Alexander J. Travis, VMD, PhD)

Introduction: Routine semen analysis correctly identifies male infertility only half the time. Localization patterns of GM1 in human sperm are highly associated with capacitation and their ability to fertilize. Here, the laboratory developed Cap-Score™ Sperm Function Test was used to define a standard capacitation profile for men of proven fertility, which was then compared to a group of men seeking male fertility workups.
Methods: Two cohorts were defined: 1) normal fertile men (pregnant partner or having a child within 3 years), and 2) potential subfertile/infertile men seeking semen analysis at the Urology Group of New Jersey. Subsequent to obtaining consent, collection and liquefaction, sperm were washed, incubated, fixed and then evaluated via fluorescence microscopy to determine Cap-Score.
Results: The Cap-Score mean (μ=39) and SD (σ=7) from 41 fertile men were used to estimate the number of fertile men needed to establish a robust fertile capacitation profile. For a power analysis, an acceptable range about the mean was set at 3%, and a two-tailed t-test at the $p<0.01$ level with a probability of detecting a difference this large of 90% was applied. Results suggested that a valid standard can be established with ≥85 individuals. A preliminary fertile standard was created using 125 observations from 41 unique donors. Cap-Scores were averaged by donor and converted to z-scores ($(X-\mu)/\sigma$; X=observation, μ=39; σ=7). This transformed the μ to 0 and the σ to 1, with converted values representing the distance from the μ (mean) in units of σ (S.D.). The normal fertile standard was tested against Cap-Scores from 93 men seeking fertility exams. This cohort scored significantly below the fertile population ($p=1.6E-5$), with 27 and 38% having z-scores ≤-2 and between -1 and -2. Only 35% scored near or above the mean. These data suggest that in comparison to fertile men, many men seeking fertility exams have defects in capacitation.
Conclusions: Classic analyses provide little information on the ability of a semen sample to fertilize. Capacitation is required for fertilization and can be assessed using GM1 localization. A robust capacitation profile can be defined and employed for identifying abnormalities. Remarkably, 65% of men questioning their fertility had z-scores ≤-1 versus an expected result of 16%. Combining the Cap-Score™ Sperm Function Test with traditional analyses should prove valuable in diagnosing male infertility.
Funding: Funded by Androvia LifeSciences.

141

SONOGRAPHIC MEASUREMENT OF VISCERAL ADIPOSE TISSUE (VAT) WAS SUPERIOR TO OTHER ANTHROPOMETRIC MEASURES OF BODY FAT AS A PREDICTOR OF SPERM COUNT, MOTILITY AND DNA FRAGMENTATION (DFI).

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(Presented By: Kevin Quach, MSc, CCRP)

Introduction: Spermatogenesis is highly complex and affected by numerous intrinsic and extrinsic factors. Previous studies have shown that obesity has an impact on sperm production. VAT has been studied as a predictor of gestational diabetes, but its impact on gametogenesis has not been previously reported. For this study, we assessed for a correlation between VAT and various sperm parameters, and compared it as a predictor to other anthropometric measures of body fat.
Methods: This study included 91 male patients referred with their partners to a university-affiliated infertility clinic. After giving informed consent, each had an adiposity scan to measure VAT and subcutaneous adipose tissue (SCAT) by abdominal ultrasound. We evaluated for correlations between various anthropometric measures of body fat, including: VAT, SCAT, BMI and waist-hip-ratio (WHR) with semen analysis (SA) parameters and DNA fragmentation index (DFI). A pairwise analysis was conducted between these parameters.
Results: VAT had a significant negative correlation with sperm count [-26.0% (CI: -48.0%, -1.0%)] and motility [-17.2% (CI: -41.0%, -0.8%)], and positive correlation with DFI [+23.1 (CI: 3.9%, 47.0%)], while BMI had a significant negative correlation with sperm count and motility [-32.0 (CI: -56.1%, -2.9%) and -18.5% (-46.3%, -1.6%), respectively], but no correlation with DFI. SCAT had a negative correlation with sperm morphology [-29.6 (CI: -52,2%, -3.1%)] WHR did not correlate with any SA parameters or DFI. There was no correlation between semen volume and any anthropometric parameter.
Conclusion: Both BMI and VAT, but not WHR or SCAT significantly correlated with both sperm count and motility. Interestingly, VAT, but not BMI, also correlated with DFI measurement. Our findings suggest a strong metabolic impact of VAT on both sperm count and motility, together with the level of sperm DNA oxidative damage. These findings emphasize the close relation between metabolic status and male gametogenesis. The relevance of VAT is strengthened due to its reversibility by life style modifications. Sonographic scanning for VAT has potential to be a useful addition to the initial male infertility evaluation and follow up.

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142

IDENTIFICATION OF GATA4-DEPENDENT TARGET GENES IN SERTOLI CELLS BY KNOCKDOWN IN MSC-1 CELLS

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(Presented By: Marie France Bouchard, PhD)

GATA4 is an essential transcriptional regulator required for initiating the development of the gonadal primordium, proper gonadal differentiation at the time of sex determination, and fertility in both males and females. While many mouse knockout models have identified these key physiological roles, much less is known about its mechanism of action; that is, the network of genes that are directly or indirectly regulated by GATA4. We and others have recently identified GATA4 as a critical regulator of Leydig cell steroidogenesis. Knockdown of GATA4 in the MA-10 Leydig cell line blocked steroid production by inhibition of multiple genes involved in testosterone biosynthesis. GATA4 is also prominently expressed in testicular Sertoli cells. In the fetal testis, GATA4 regulates Sertoli cell differentiation and therefore testis morphogenesis. In the postnatal testis, GATA4 is required for male fertility. How GATA4 contributes to these physiological roles, however, has yet to be fully understood. We have now investigated the effects of knocking down GATA4 in the MSC-1 cell line to gain better insights into its mechanism of action in the Sertoli cell lineage. We used small interfering ribonucleic acids (siRNAs) directed against the GATA4 coding sequence to generate GATA4 depleted MSC-1 Sertoli cells. Microarray screening analysis of the transcriptome isolated from these cells showed a remarkable change in the expression of several transcripts. We then sought to identify a subset of relevant modulated genes for a more in-depth investigation. We systematically processed the data using pathway analysis software to identify pathways or cellular processes affected by GATA4 depletion. More than 80 genes were thus classified as markedly up- or downregulated in these cells. Based on these analyses, nearly 30 genes were assayed by qPCR to confirm or invalidate their up- or downregulated status in GATA4-depleted cells. Remarkably, genes involved in cell junction signaling were noticeably downregulated while genes involved in apoptosis were significantly upregulated in GATA4-depleted cells. Phagocytosis and prostaglandin synthesis were other pathways that were markedly affected by the knockdown of GATA4. The identification of new GATA4 gene targets in Sertoli cells helps to clarify the mechanism of action of this important transcription factor in the Sertoli cell and more broadly in the testis.
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143

MEF2 REGULATES NRF2 GENE EXPRESSION IN MOUSE MA-10 LEYDIG CELLS

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(Presented By: Nicholas Robert)

Leydig cells are the main producers of testosterone, a hormone essential for spermatogenesis and for the development of male sexual characteristics. Steroidogenesis, however, produces a significant amount of reactive oxygen species (ROS), which in turn disrupt testosterone production. Our lab has recently identified members of the Myocyte Enhancer Factor 2 (MEF2) family of transcription factors as regulators of the Gsta (glutathione S-transferase a) class of genes involved in the regeneration of glutathione in Leydig cell, a process essential for ROS elimination and thus maintenance of testosterone production. Leydig cells also express the transcription factor NRF2 (NFE2L2), which is a well-characterized master regulator of detoxification enzyme-encoding genes in various cells. Since both MEF2 and NRF2 are expressed in Leydig cells and are both involved in detoxification mechanisms, we tested the possibility that MEF2 and NRF2 might operate in a common regulatory cascade. Transcriptomic analyses of MEF2-depleted (siRNA-mediated knockdown) MA-10 Leydig cells revealed a decrease in Nrf2 expression. This was further validated by qPCR where a reduction of 30% in Nrf2 mRNA levels was observed in MA-10 cells depleted in MEF2 factors. Conversely, overexpression of MEF2 in MA-10 Leydig cells led to a 1.3 fold increase in endogenous Nrf2 mRNA levels. A -1308 bp fragment of the mouse Nrf2 promoter was isolated, fused to a luciferase reporter and used in functional promoter studies. In transient transfection assays in MA-10 cells, we found that the -1308 bp Nrf2 reporter was activated by ~1.8 fold in the presence of MEF2. Deletion to -1261 bp, however, abolished MEF2 responsiveness. This indicates that the MEF2 responsive element(s) are located between -1308 and -1261 bp. In silico analyses of this 47 bp region of the Nrf2 promoter revealed the presence of a consensus MEF2 binding site at -1270 bp. Studies are currently underway to assess MEF2 binding to this MEF2 element in the Nrf2 promoter. In conclusion, our present data indicate that MEF2 is a regulator of Nrf2 gene expression in MA-10 Leydig cells and as such position MEF2 upstream of NRF2 in a regulatory cascade controlling ROS detoxification in these cells.
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144

IMMUNOHISTOCHEMICAL EXPRESSION PATTERNS OF FACTORS ASSOCIATED WITH DEVELOPMENT OF PORCINE TESTIS TISSUE

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(Presented By: Awang Hazmi Junaidi, DVM, MVSc)

Testis development continues throughout the fetal and early postnatal periods and involves complex processes that remain poorly understood. In this study, we evaluated the immunohistochemical expression patterns of a number of intrinsic factors that are potentially associated with the development of testis tissue including the sex determining region Y (Sry), SRY-box 9 (SOX9), fibroblast growth factor 9 (FGF9), prostaglandin D2 synthase (PGD2) and anti Mullerian hormone (AMH). Immunohistochemistry (using horseradish peroxidase assay – HRP) was performed on testis tissue samples of prenatal (68 days and 82 days of fetal ages, n=3/age), neonatal (1, 2, 3 and 4 wk of age, n=5/age), prepubertal (9 and 14 wk of age, n=5/age) and sexually mature (n=4) Yorkshire-cross pigs. We observed that Sry was expressed in the prenatal testes and in the neonatal testes until 3 wk of age. The expression of SOX9 was persistently observed in all age groups, mostly in Sertoli and germ cells. Spatial distribution of FGF9 changed over time; in the prenatal and neonatal testes the expression was observed within the seminiferous cords (i.e., in Sertoli cells and germ cells) while at 9 wk of age and later ages the expression was observed in the interstitial tissue (i.e., in Leydig cells). Expression of the AMH was observed in the cytoplasm of Sertoli cells and in a diffused form in the seminiferous cords of prenatal testes and persisting up to 4 wk of age in postnatal testes. The diffused form of AMH expression was diminished at 9 wk and the expression became limited to Sertoli cells at 14 wk of age, and was completely absent in the mature testes. Similarly, the expression pattern of PGD2 was localized in the pre- and postnatal Sertoli cells and the diffused form persisted until 9 wk of age before the expression shifted to the interstitial tissue at 14 wk and later ages. These observations indicate that the protein expression patterns of intrinsic factors associated with porcine testis development are stage/age dependent and these patterns may be important for the developmental changes of the testis.

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145

OPTIMIZATION OF PRIMARY CULTURE CONDITIONS FOR NEONATAL PORCINE GONOCYTES

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University of Saskatchewan
(Presented By: Awang Hazmi Junaidi, DVM, MVSc)

Gonocytes are the only germ cells present in the fetal and neonatal testes and, by giving rise to spermatogonial stem cells, are essential for upcoming spermatogenesis. Efficient culture of gonocytes provides a unique opportunity for the study, proliferation and manipulation of these stem cell precursors. We examined a number of culture conditions that potentially affect the proliferation and development of neonatal porcine testis cells. Testes (n=80) from one wk old Yorkshire−cross piglets were collected for isolation of testis cells using a three−step enzymatic digestion (designed to yield ~40% gonocytes, Yang et al., 2010), with or without subsequent gonocyte enrichment (yielding ~80−90% gonocytes, Yang and Honaramooz, 2011). The cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum in 6−well plates. Effects of several culture conditions were examined on the morphology of testis cells using cytology, immunocytochemistry and live−cell imaging. The culture conditions included gonocyte composition (40% vs. 80% vs. 90%), incubation temperature (35ºC vs. 37ºC, with 5% CO2), cell seeding density (1.0 × 10^5 vs. 3.0 × 10^5 vs. 5.0 × 10^5 cells/well) and media changing regimen (every 2 days vs. no changing for up to 7 days). We observed that optimal cell growth (i.e., 90% confluency in ~6 days) was achieved when testis cells with a gonocyte composition of ~40% and a cell seeding density of 3.0 × 10^5 cells/well were used. While culturing the cells at 35°C or 37°C were equally effective, the lower incubation temperature resulted in delayed growth of cells. The results also indicated that a critical minimum ratio of somatic cells are required for settlement of gonocytes, and that gonocytes can proliferate even in a basic culture media, without added exogenous growth factors. We also observed that in the first 2 days of culture, gonocytes remained mostly floating in the media and gradually settled over the next 5 days. Consequently, changing the media once a week (vs. the conventional every 2 days) led to a significant increase in the colonization and proliferation of gonocytes, perhaps by minimizing the loss of these floating gonocytes. The optimized primary culture conditions outlined in this study can be used to improve in vitro maintenance and proliferation of neonatal porcine gonocytes.

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147

ACTIVATION OF NOTCH SIGNALING IN SERTOLI CELLS IN VIVO IS DEPENDENT UPON THE PRESENCE OF GERM CELLS.

Thomas Garcia, PhD[1]; Parag Parekh, PhD[2]; Sean Kow, BS[3]; and Marie-Claude Hofmann PhD[2]
[1]University of Houston-Clear Lake; [2]UT MD Anderson Cancer Center; [3]Texas Tech University Health Sciences Center School of Medicine
(Presented By: Thomas Garcia, PhD)

Recently, we demonstrated through complementary gain- and loss-of-function mouse models that NOTCH signaling activation in Sertoli cells results in downregulation of the key germ cell niche factor Gdnf, which is required for proper maintenance and proliferation of the spermatogonial stem cell population. We also ascertained through mRNA and protein expression analyses that germ cells are the ligand expressing cells in vivo and that germ cells in vitro are capable of activating NOTCH signaling in Sertoli cell cultures. To demonstrate germ cell-dependent activation of NOTCH signaling in Sertoli cells in vivo, we ablated the germ cell compartment in mice using the sterilizing

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agent busulfan and isolated Sertoli cells from these mice and controls using yellow fluorescent protein (RosaYFP) expression specific to Sertoli cells (using Amh-cre). The results demonstrated that expression of the canonical NOTCH target genes Hey1 and HeyL were significantly downregulated in Sertoli cells. Concomitantly, expression of Gdnf was significantly upregulated, consistent with previous in vitro and in vivo experiments in Sertoli cells whereby NOTCH signaling was inhibited or ablated. Taken together, these results support the notion that germ cells are the ligand expressing cells capable of activating NOTCH signaling in Sertoli cells. Furthermore, these results provide further evidence to support the notion that NOTCH signaling in Sertoli cells may be functioning as a negative feedback mechanism that germ cells may use to control their own numbers. Further study is required to better understand the specific germ cell population potentially driving this NOTCH-dependent feedback inhibition.

148

ABLATION OF RBPJ RESCUES EARLY PRO-SPERMATOGONIA LOSS FROM ECTOPIC ACTIVATION OF NOTCH SIGNALING IN MOUSE SERTOLI CELLS

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(Presented By: Thomas Garcia, PhD)

Stem cells are influenced by their surrounding niche microenvironment. In the testis, Sertoli cells are the key niche cells directing the fate of germ cells, including spermatogonial stem cells (SSCs). Our previous studies with Sertoli-cell specific Notch gain- and loss-of-function mouse models demonstrated that Notch signaling in Sertoli cells is crucial for the proper regulation of germ cell numbers by limiting GDNF expression. In our gain-of-function model constitutively expressing NICD (the activated form of the Notch receptor), fetal pro-spermatogonia underwent aberrant early differentiation, leading to their apoptosis and Sertoli cell-only syndrome shortly after birth. In contrast, in our loss-of-function model that lacked a functional RBPJ downstream of NICD, the number of fetal gonocytes was unaffected while the number of GFRA1+ and PLZF+ spermatogonia after birth was increased, leading to a significant increase in spermatocytes and spermatids, and in 20% of knockouts, testicular atrophy with tubule lithiasis. Since NICD can have non-canonical, RBPJ-independent functions, we postulated that generating a double-mutant mouse model with constitutive expression of NICD in a background of RBPJ ablation in Sertoli cells would reveal whether non-canonical Notch signaling exists in Sertoli cells. Following phenotype analysis, we found these double mutant mice displayed a phenotype indistinguishable to RBPJ single null mice. These results reveal that Notch signaling in Sertoli cells functions solely through the NICD/RBPJ canonical axis.

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ANDROLOGY
Contents

1 Schedule at a Glance
5 President's Welcome
5 Past Presidents
7 ASA Officers
8 General Meeting Information
10 Message from the Program Co-Chairs
11 Emil Steinberger Memorial Lecture Award
12 AUA Lecture
12 Outstanding Trainee Investigator Award
13 Distinguished Andrology Award
14 Distinguished Service Award
15 Matthew P. Hardy Young Andrologist Award
16 Thank You to Donors & Sponsors
18 Educational Needs & Objectives
19 Accreditation Statement
20 Schedule of Events
27 Speaker Abstracts
35 Oral Abstracts
41 Poster Session I
52 Poster Session II
62 Index of Abstract Authors
67 Abstract Full Text
130 Committee Listing