XXVth North American Testis Workshop

"Lifelong Cell-Cell Interactions in the Testis: A Driver for Male Fertility"

April 3 - 6, 2019

The Ritz-Carlton Chicago | Chicago, IL

WELCOME TO THE XXVTH NORTH AMERICAN TESTIS WORKSHOP

Welcome to the XXVth North American Testis Workshop (NATW). Understanding the cellular, biochemical and molecular processes in the testis that underpin male fertility will contribute to the early diagnosis, prevention and treatment of infertility as well as to the development of a reversible male contraceptive. The NATW is the only conference dedicated solely to basic and clinical studies of the normal and pathological, fertile and infertile testis. The Workshop has been held every two years since 1972, every time at a different location. This year we meet in Chicago, the third largest city in the US, located on the shores of Lake Michigan, an international hub for commerce, industry, finances, technology, telecommunications, and transportation.

The Workshop provides an extremely important venue to disseminate the latest developments in testis biology and to establish collaborations needed to provide solutions to the pressing needs in male reproductive health. The XXVth Workshop's theme is "Lifelong cell-cell interactions in the testis: a driver for male fertility" and aims to continue the well-established tradition of providing an international forum for the presentation and discussion of current results by leaders in this field, and for fostering the inclusion and career development of young scientists, women, and underrepresented minorities.

This year's program includes four major lectures (the keynote address and three benchmark lectures), and 15 invited talks that are grouped into five sessions as follows: Cell-cell interactions in testis formation; Germ cell differentiation in response to local and endocrine factors; Somatic cell function in response to local factors; Testicular function in response to man-made chemicals; and Pathogen effects on testis function. After the invited talks, sessions will end with short talks selected from abstracts submitted by young investigators.Poster sessions are included, with allPosters displayed throughout the meeting.

We would like to thank members of the various committees and people who provided time and effort to the design, development, organization and funding of the Workshop. These include the Program, Abstract Review and Executive Committees (listed below). We are grateful to the following organizations that provided essential financial support for the meeting: The Eunice Kennedy Shriver National Institute of Child Health & Human Development, the Male Contraception Initiative, the Society for the Study of Reproduction, and IASO BioMed Inc. We would also like to thank Ms. Donna Rostamian and her team at WJ Weiser & Associates for the management and taking care of all the details needed to make this meeting feasible.

The Testis Workshop exists and works because of the many excellent scientists who attend and contribute to each meeting. We hope that the meeting will strengthen collaboration and information sharing among our community and help translate our research into real life changes for millions of men suffering from male reproductive disorders and infertility. Thank you all for participating and we hope you enjoy the meeting and Chicago venue.

Vassilios Papadopoulos, PhD, Chair of the Program Committee
Wei Yan, MD, PhD, Vice-Chair of the Program Committee

TESTIS WORKSHOP COMMITTEES

Program Committee

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Jon Oatley, PhD
Elizabeth Snyder, PhD

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Jacquetta Trasler, MD, PhD
Robert Viger, PhD
William Wright, PhD
Wei Yan, MD, PhD
Barry Zirkin, PhD

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FACULTY LISTING

XXVth North American Testis Workshop
"Lifelong Cell-Cell Interactions in the Testis: A Driver for Male Fertility"
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Laval University

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Duke University Medical Center

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University of Southern California

Tony De Falco, PhD
Cincinnati Children's Hospital Medical Center

Ina Dobrinski, DVM, PhD
University of Calgary

Ellen Goossens, PhD
Vrije Universiteit Brussels

Michael D. Griswold, PhD
Washington State University

Barbara F. Hales, PhD
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NICHD, NIH

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University of Toronto

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Monash University and Hudson Institute of Medical Research

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Ludwig-Maximilian-University

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Utah State University

Jon M. Oatley, PhD
Washington State University

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University of Southern California School of Pharmacy

J. Richard Pilsner, PhD, MPH
University of Massachusetts Amherst

Kotaro Sasaki, MD, PhD
University of Pennsylvania

Elizabeth Snyder, PhD
Rutgers University

Jan-Bernd Stukenborg, PhD
Karolinska Institutet and University Hospital

Guy Tanentzapf, PhD
University of British Columbia

Kenneth S. K. Tung, MD
University of Virginia

Saguna Verma, PhD
University of Hawaii

William Wright, PhD
Johns Hopkins University

Wei Yan, MD, PhD
University of Nevada

GENERAL MEETING INFORMATION

HOTEL INFORMATION

The Ritz-Carlton Chicago
160 E. Pearson Drive
Chicago, IL 60611
Main: (800) 542-8680
Website: ritzcarlton.com/en/hotels/chicago

WELCOME TO CHICAGO

The city that feels like home, which is a city of neighborhoods, Chicago is wholly unique and no matter where you go, each part has its own draw: the buzzing Loop downtown and upscale River North, artsy Wicker Park, and scholarly Hyde Park. You can visit world-class museums, dine at one of our many Michelin-star restaurants or catch a show in one of over 200 theaters. No matter what you love to do, you'll feel right at home doing it in Chicago.

One of Chicago's most top attractions is Navy Pier, only a 10 minute drive from the Ritz-Carlton! First opening in 1916, Navy Pier has been a famous destination for over 100 years. Offering visitors a 360-degree view of Chicago, you can take a ride on the iconic Centennial Wheel, which soars nearly 200 feet in the air. Another breathtaking experience is the Crystal Gardens, an indoor, one-acre botanical garden housed within a six story glass atrium. The garden holds over 80 live palm trees, foliage, and dancing leapfrog fountains. There is also a vast array of restaurants and live entertainment lined up across Navy Pier. To plan your trip to Navy Pier and see all it has to offer, visit www.navypier.org.

There are plenty of other things to do in Chicago, including tours, spectator sports, shopping and other attractions. Our nightlife doesn't stop either with plenty of bars and nightclubs, live music and comedy clubs. During the 19th century, Chicago was a major hub for the shipping industry; present day Chicago is better known for its vibrant music scene—the city played a seminal role in the emergence of jazz and blues, and its symphony orchestra is a standout—but its history as a major port city is still reflected in Chicago's vibrant waterfront district. Design and architecture buffs should plan a trip for some of the country's most cutting-edge buildings and monuments such as Cloud Gate, the giant reflective bean sculpture found in Millennium Park along with Crown Fountain, which contains two digital, 50-foot towers projecting facial images of Chicago citizens that spout water into a shallow reflecting pool that visitors can walk through. Millennium Park also features state-of-the-art collections of architecture, landscape design, and art. Take a walk through the Lurie Garden, a 3.5-acre urban sanctuary with 15-foot-high hedges and hardwood footbridges over shallow water. You can learn more about Millennium Park at www.choosechicago.com.

TESTIS WORKSHOP REGISTRATION DESK HOURS

Location: Grand Foyer

Wednesday, April 3, 2019	6:00 p.m. - 8:30 p.m.
Thursday, April 4, 2019	7:00 a.m. - 6:00 p.m.
Friday, April 5, 2019	7:00 a.m. - 6:00 p.m.
Saturday, April 6, 2019	7:15 a.m. - 12:00 p.m.

TESTIS WORKSHOP WELCOME RECEPTION

Date:	Wednesday, April 3, 2019
Time:	8:15 p.m. - 9:30 p.m.
Location:	Grand Foyer
Cost:	One ticket included in registration fee

TESTIS WORKSHOP SOCIAL EVENT

Date:	Thursday, April 4, 2019
Time:	6:30 p.m. - 8:30 p.m.
Location:	Lou Malnati's Pizzeria 1120 N State Street, Chicago, IL 60610
Cost:	Advanced ticket purchase required to attend

TRAVEL AND TRANSPORTATION

Airport Information
O'Hare International Airport (ORD) is approximately 18 miles from The Ritz-Carlton Chicago or 30-60 minutes by car. Chicago Midway International Airport (MDW) is approximately 12 miles from The Ritz-Carlton Chicago or 30-45 minutes by car.

Taxi Cab Services
Several taxi companies operate at O'Hare International Airport. Available taxis are located at the lower level curb outside of the Baggage Claim area at each terminal. Taxi rates for a one-way transfer to downtown Chicago from O'Hare International Airport cost approximately $30-$40. Taxi rates for a one-way transfer to downtown Chicago from Midway International Airport cost approximately $28-$30.

Rental Car Information
Avis® Rent-A-Car is the official rental car company for the ASA 44th Annual Conference. For reservations, please call (800) 331-1600, and use the code "J901055" to receive the discounted rates.

Public Transportation
Both Chicago airports offer easy access to the Chicago Transit Authority's "L" System. From O'Hare International Airport, a one-way fare downtown on the Blue Line costs $2.25. From Midway Airport, a one-way fare downtown on the Orange Line costs $2.25. Please visit the following link for detailed directions: www.transitchicago.com/travel_information/trip_planner.aspx

Parking
ASA is happy to offer discounted parking at The Ritz-Carlton Chicago at the rate of $33 for self parking and $71 for valet parking.

PROGRAM SCHEDULE

The XXVth North American Testis Workshop
"Lifelong Cell-Cell Interactions in the Testis: A Driver for Male Fertility"

April 3 - 6, 2019 | The Ritz-Carlton Chicago

Chair: Vassilios Papadopoulos, PhD & Vice Chair: Wei Yan, MD, PhD

All sessions will be located in ***The Ritz-Carlton Ballroom*** unless otherwise noted. | Speakers and times are subject to change.

WEDNESDAY, APRIL 03, 2019

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

7:00 p.m. - 7:15 p.m. **Welcome and Opening Remarks**
Program Chair:
Vassilios Papadopoulos, PhD
University of Southern California
School of Pharmacy

7:15 p.m. - 8:15 p.m. **KEYNOTE ADDRESS:**
Antigens and Blood - Testis Barrier
Kenneth S. Tung, MD
University of Virginia

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

THURSDAY, APRIL 04, 2019

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

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

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

8:00 a.m. - 8:05 a.m. **Chair and Introduction**
Chair: William Wright, PhD
Johns Hopkins University

8:05 a.m. - 8:55 a.m. **Study of the Testicular Microenvironment In Vitro**
Jan-Bernd Stukenborg, PhD
Karolinska Institutet and University Hospital

SESSION I: CELL-CELL INTERACTIONS IN TESTIS FORMATION

8:55 a.m. - 9:00 a.m. **Chair and Introduction to Session I**
Session Chair: Marie-Claude Hofmann, PhD
MD Anderson Cancer Center

9:00 a.m. - 9:40 a.m. **Testicular Organoids to Study Cell-Cell Interactions in the Mammalian Testis**
Ina Dobrinski, DVM, PhD
University of Calgary

9:40 a.m. - 10:20 a.m. **Germline Specification in Primates and its Reconstitution In Vitro**
Kotaro Sasaki, MD, PhD
University of Pennsylvania

THURSDAY, APRIL 04, 2019 (continued)

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

10:40 a.m. - 11:20 a.m. **Genetic Networks that Act in Somatic Cells of the Testis to Mediate Germ Cell Development**
Guy Tanentzapf, PhD
University of British Columbia

11:20 a.m. - 11:35 a.m. **Short Talk #1**
Single-cell Transcriptomes of Prospermatogonia Reveal Heterogeneity in the RA Response in the Neonatal Mouse Testis
Presented By: Anukriti Singh, B.Tech
University of Texas at San Antonio

11:35 a.m. - 11:50 a.m. **Short Talk #2**
Directing Human Induced Pluripotent Stem Cells Differentiation Towards Leydig and Adrenal Cells
Presented By: Lu Li, PhD
University of Southern California

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

SESSION II: GERM CELL DIFFERENTIATION IN RESPONSE TO LOCAL AND ENDOCRINE FACTORS

1:10 p.m. - 1:15 p.m. **Chair and Introduction to Session II**
Session Chair: Elizabeth Snyder, PhD
Rutgers University

1:15 p.m. - 1:55 p.m. **Positional Influence on Establishment of the Foundational Spermatogonial Stem Cell Pool**
Jon M. Oatley, PhD
Washington State University

1:55 p.m. - 2:35 p.m. **Potential Use of Stem Cells for Fertility Preservation**
Clifford L. Librach, MC, FRSCS(C), FACOG
University of Toronto

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

2:55 p.m. - 3:35 p.m. **Human Testis Organoid Formation**
Ellen Goossens, PhD
Vrije Universiteit Brussels

3:35 p.m. - 3:50 p.m. **Short Talk #3**
Single-cell RNA Sequencing Reveals Novel Markers of Stem/progenitor Spermatogonia in Higher Primates
Presented By: Sarah Munyoki, BA
University of Pittsburgh

PROGRAM SCHEDULE

THURSDAY, APRIL 04, 2019 (continued)

3:50 p.m. - 4:05 p.m. **Short Talk #4**
Dynamic Subcellular Membrane Lipid Remodeling in Hormone-induced Leydig Cell Steroidogenesis
Presented By: Sathvika Venugopal, PhD
McGill University Health Centre

4:05 p.m. - 6:00 p.m. **Poster Session**

6:30 p.m. - 8:30 p.m. **Testis Workshop Social Event***
Location: Lou Malnati's Pizzeria
1120 N State Street, Chicago, IL 60610
**Advanced ticket purchase required*

FRIDAY, APRIL 05, 2019

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

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

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

8:00 a.m. - 8:05 a.m. **Chair and Introduction**
Chair: Katherine Loveland, PhD
Monash University and Hudson Institute of Medical Research

8:05 a.m. - 8:55 a.m. **Establishing and Replacing Sertoli Cells in the Testis**
Blanche Capel, PhD
Duke University Medical Center

SESSION III: SOMATIC CELLS FUNCTION IN RESPONSE TO LOCAL FACTORS

8:55 a.m. - 9:00 a.m. **Chair and Introduction to Session III**
Session Chair: Mirella Meyer-Ficca, PhD
Utah State University

9:00 a.m. - 9:40 a.m. **Interactions Between Sertoli Cells and Germ Cells Govern the Cycle of the Seminiferous Epithelium**
Michael D. Griswold, PhD
Washington State University

9:40 a.m. - 10:20 a.m. **Peritubular Cells of the Human Testis: Prostaglandin E2 (PGE2) and More**
Artur Mayerhofer, MD
Ludwig-Maximilian-University

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

10:40 a.m. - 11:20 a.m. **Role of the Interstitial Cells in Spermatogonial Stem Cell Development**
Tony De Falco, PhD
Cincinnati Children's Hospital Medical Center

FRIDAY, APRIL 05, 2019 (continued)

11:20 a.m. - 11:35 a.m. **Short Talk #5**
The Role of Rna Binding Protein Adad2 in Male Meiosis
Presented By: Lauren Chukrallah
Rutgers the State University of New Jersey

11:35 a.m. - 11:50 a.m. **Short Talk #6**
Unravelling the Role of Trim28 in Spermatogenesis
Presented By: Joel Heng Loong Tan, BSc (Hons)
Institute of Molecular and Cell Biology (IMCB)

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

11:50 a.m. - 1:10 p.m. **Testis Workshop Executive Committee Meeting**

SESSION IV: TESTICULAR FUNCTION IN RESPONSE TO MAN-MADE CHEMICALS

1:10 p.m. - 1:15 p.m. **Chair and Introduction to Session IV**
Session Chair: Benson T. Akingbemi, DVM, PhD
Auburn University

1:15 p.m. - 1:55 p.m. **Effects of Flame Retardants on Testicular Function**
Barbara F. Hales, PhD
McGill University

1:55 p.m. - 2:35 p.m. **Phthalate Exposure on Sperm Epigenetics and Early-life Development in Man and Mouse**
J. Richard Pilsner, PhD, MPH
University of Massachusetts Amherst

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

2:55 p.m. - 3:35 p.m. **Effects of Endocrine Disruptor Mixtures on Male Reproduction**
Martine Culty, PhD
University of Southern California

3:35 p.m. - 3:50 p.m. **Short Talk #7**
Functional Role of the Copper Transporter 1 Protein in Spermatogenesis and in Cisplatin-induced Testicular Injury
Presented By: Rashin Ghaffari, BS
The University of Texas at Austin

3:50 p.m. - 4:05 p.m. **Short Talk #8**
HIPK4 Is Essential for Murine Spermiogenesis
Presented By: J. Aaron Crapster, PhD
Stanford University

4:05 p.m. - 6:00 p.m. **Poster Session**

PROGRAM SCHEDULE

SATURDAY, APRIL 06, 2019

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

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

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

8:00 a.m. - 8:05 a.m. **Chair and Introduction**
Chair: Janice L. Bailey, PhD
Laval University

8:05 a.m. - 8:55 a.m. **Testis & Plastics: 25+ Years of the Estrogen Hypothesis**
Kim Boekelheide, MD, PhD
Brown University

SESSION V: PATHOGEN EFFECTS ON TESTIS FUNCTION

8:55 a.m. - 9:00 a.m. **Chair and Introduction to Session V**
Session Chair: Dolores J. Lamb, PhD, HCLD
Cornell University

9:00 a.m. - 9:40 a.m. **The Impact of ZIKA Infection on Male Fertility and the Role of Vaccination**
Gary Kobinger, PhD
Laval University

9:40 a.m. - 10:00 a.m. **Break**

10:00 a.m. - 10:40 a.m. **Newly Emerging Sexually Transmitted Viruses: Testicular Defense Systems and Clinical Implications**
Saguna Verma, PhD
University of Hawaii

10:40 a.m. - 11:20 a.m. **The Immunology of Male Reproduction**
Mark Hedger, PhD
Hudson Institute and Monash University

11:20 a.m. - 11:35 a.m. **NIH**
Travis Kent, PhD
NICHD, NIH

11:35 a.m. - 11:50 a.m. **Concluding Remarks and Acknowledgements**
Wei Yan, MD, PhD
University of Nevada

11:50 a.m. - 12:00 p.m. **Announcement of the XXVIth North American Testis Workshop**
Wei Yan, MD, PhD
University of Nevada

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

SPEAKER ABSTRACTS

WEDNESDAY, APRIL 3, 2019
7:15 p.m. - 8:15 p.m.

KEYNOTE ADDRESS
Antigens and Blood - Testis Barrier
Kenneth S. Tung, MD
Professor, Department of Pathology, and member, B Carter Center for Immunology Research, University of Virginia, Charlottesville, VA 22108.

Current paradym: Testis antigens (Ag), hidden behind tissue barriers, are not protected by systemic immune mechanisms. Cancer-testis Ag (CTA) are highly immunogenic as cancer vaccine Ag. However, these assumptions are not validated.
Two FOXP3+ T regulatory cells (Treg) control autoimmune disease: Thymus-derived Treg (tTreg) prevents disease occurrence. Induced-Treg (iTreg), converted from non-Treg T cells in extra-thymic inflamed tissues, reduces ongoing disease severity/progress.
tTreg prevent autoimmunity to interstitial testis Ag. In thymus, tTreg development depends on the AIRE gene. In periphery, tTreg require continuous interaction with tissue Ag. We depleted tTreg from DEREG mice expressing transgenic FOXP3-Diphtheria toxin receptor, and detected spontaneous Ab to: Leydig cells including steroid enzymes 450scc and 17a-hydroxylase; and Stra8+ spermatogonia cells. The Ab are made by autoimmune polyendocrine syndrome patients with AIRE mutation; and human cancer vaccines utilize spermatogonial Ag. Therefore, clinical-relevant testis interstitial Ag are protected by tTreg.
tTreg also prevent autoimmunity to meiotic sperm Ag that egress seminiferous tubule (ST). We discovered egress of lactate dehydrogenase 3 (LDH3), but not zonahesin (ZAN), from normal ST. LDH3 formed immune complexes with circulating Ab outside BTB. As contents of residual body and cytoplasmic droplet, derived from spermatids at spermiation, the egressed sperm Ag escaped Sertoli cell destruction, and reached interstitial space. Importantly, Treg-depleted DEREG mice produced Ab to LDH3 but not to ZAN. Therefore, meiotic sperm Ag that egress normal ST are also protected by tTreg.
iTreg prevent immune response to hidden sperm Ag exposed in inflamed epididymis after vasectomy (Vx). Vx mice resisted autoimmune orchitis induction for 3 months unless Treg-depleted within 7 days post-Vx. Treg-depleted Vx mice produced Ab to ZAN but not to LDH3. Therefore, ZAN and other hidden sperm Ag, unprotected by tTreg, induce protective iTreg when exposed.
Conclusion: Complete testis antigen sequestration is a false concept. Instead, testis Ag communicate with the immune system to maintain tTreg, or to invoke iTreg, for effective systemic immune protection.

THURSDAY, APRIL 4, 2019
8:05 a.m. - 8:55 a.m.

BENCHMARK LECTURE I
Study of the Testicular Microenvironment In Vitro
Jan-Bernd Stukenborg, PhD
NORDFERTIL Research Lab Stockholm; Department of Women's and Children's Health; Karolinska Institutet and University Hospital, Stockholm, Sweden

Male fertility is dependent on the spermatogenic process leading to the production of fertile male gametes. It comprises the proliferation of spermatogonial stem cells (SSCs), meiotic division, and the differentiation and maturation of spermatids. The fact that spermatogenesis is a continuous process which relies on stem cells offers a male the opportunity to generate sperm until late in life as long as the SSC population is not lost or destroyed. Throughout recent decades, different strategies, including numerous in vitro culture setups, have been employed to explore the spermatogenic progress in detail. Despite this understanding of this highly complex process, which is influenced by endocrine and paracrine actions of the somatic testicular cell compartment (e.g. Sertoli and Leydig cells), is still inadequate. This lack of detailed knowledge of mammalian and especially human gametogenesis also results in a paucity of treatment options for patients, who cannot produce sperm and are at the risk of becoming infertile, due to their medical treatment or disease itself. This highlights the need for novel strategies to both understand the process of human spermatogenesis and develop *ex vivo* approaches to differentiate mature gametes from immature germ cells or even pluripotent stem cells.
The successful production of murine sperm *in vitro* using testicular explant culture conditions was first reported in 2011 by Sato and colleagues. The described culture conditions however lack requirements enabling controlled monitoring of endo- and paracrine pathways needed to create a robust model to study specific aspects crucial to the spermatogenic process (e.g. SSC renewal, SSC niche formation, and BTB formation). Novel cell-culture methodologies established recently in other fields of medical research, for example testicular organoids, might provide new tools for more defined research approaches regarding gametogenesis and its failures. Organoids are small cell aggregates similar to organs found in vivo, but generated from single cell suspension containing stem cells. These organ-like structures are also defined by their functionality, which makes them a valuable research model and most likely a useful clinical tool in future.

THURSDAY, APRIL 4, 2019
9:00 a.m. - 9:40 a.m.

SESSION I: CELL-CELL INTERACTIONS IN TESTIS FORMATION
Testicular Organoids to Study Cell-Cell Interactions in the Mammalian Testis
Ina Dobrinski, DVM, PhD
University of Calgary, Calgary, AB, Canada

In recent years, there has been a surge of interest in three-dimensional (3D) organoids. 3D organoids were reported from both pluripotent stem cells and primary cells. Organoids bear resemblance to the primary tissue in vivo and can serve as an intermediary between animal models and conventional 2D culture models. Organoids have widespread applications such as the study of tissue development, disease modeling, and high throughput drug and toxicity screening. We have developed a 3D testicular organoid system for the study of cell-cell interactions in the testicular microenvironment. Although long-term culture of testicular cells is established in rodents using 2D systems, these culture systems often fail to reflect the complex multi-cellular interactions and signaling found in-vivo. We showed previously, that pre-pubertal porcine testicular cells can form seminiferous tubule-like structures in vitro, and support de novo morphogenesis of functional testis tissue after grafting to mouse hosts. We now employed a microwell centrifugal forced aggregation approach to establish multicellular 3D testicular organoids from pre-pubertal porcine, mouse, macaque and human testis cells. The organoids consist of germ cells, Sertoli cells, Leydig cells, peritubular myoid cells and endothelial cells with cell associations similar to those in vivo, including a distinct seminiferous epithelium and interstitial compartment separated by a basement membrane. Aspects of somatic cell as well as germ cell function are conserved and representative of the testis in vivo. Organoids can be preserved by vitrification and generated from cryopreserved testis cells. Taken together, this testicular organoid system recapitulates the 3D organization of the mammalian testis and provides an in vitro platform for studying cellular interactions in testicular development and function, and for screening drug toxicity in a cellular context representative of the testis in vivo.
Supported by NIH/NICHD HD091068-01

SPEAKER ABSTRACTS

THURSDAY, APRIL 4, 2019
9:40 a.m. - 10:20 a.m.

SESSION I: CELL-CELL INTERACTIONS IN TESTIS FORMATION
Germline Specification in Primates and its Reconstitution In Vitro
Kotaro Sasaki, MD, PhD
University of Pennsylvania

The germ cell lineage is the most fundamental component of the life cycle of the multicellular organisms, ensuring propagation of the genetic information across the generations. However, the mechanism underlying germ cell specification in primates, including humans has been unknown. By using cynomolgus monkeys as a model organism for early post-implantation development of primate embryos, we have identified the origin and the comprehensive developmental roadmap of primordial germ cells in primates. Moreover, by rigorous comparison with in-vivo dataset, we have successfully reconstituted human germ cell fate in vitro from pluripotent stem cells. Our study provides the basis for the mechanistic dissection of human germ cell specification pathway as well as the further reconstitution of the human gametogenesis in vitro.

THURSDAY, APRIL 4, 2019
10:40 a.m. - 11:20 a.m.

SESSION I: CELL-CELL INTERACTIONS IN TESTIS FORMATION
Genetic Networks that Act in Somatic Cells of the Testis to Mediate Germ Cell Development
Guy Tanentzapf, PhD
Yanina Pesch, Priya Kaur, Michael Fairchild.
Department of Cellular and Physiological Sciences
University of British Columbia, Vancouver, British Columbia

Spermatogenesis produces one of the most specialized and architecturally complex cells found in animals. Using the powerful genetic tools available in Drosophila we have been using genetic screens and candidate approaches to uncover the mechanisms that underlie sperm morphogenesis and regulate spermatogenesis. Spermatogenesis requires intricate and sustained cooperation between the soma and germline. In particular, there is extensive cell-cell signalling that takes place between the soma and germline. Through large RNAi based screens we have identified classes of genes that act in the soma to regulate the transitions between the different stages of spermatogenesis. In addition candidate approaches identified key functional roles for both occluding and gap junctions in setting up the signalling environment around the somatic and germline stem cells. While occluding junctions control the access of germline stem cells to factors that control their behaviour, gap junctions form channels between the soma and germline that are essential for stem cell differentiation and/or maintenance. A focus of our recent work is the large-scale structure/function analysis of the gap junctions proteins that are active in the testes, Inx2 in the soma and Inx4 in the germline. Based on biochemical and structural studies, we generated a collection of regulatory mutations in Innexin2 and Innexin4 to obtain mechanistic insight into gap junctions-mediated communication between the soma and germline. Our results provide insight into how the soma and the germline communicate and coordinate during spermatogenesis.

THURSDAY, APRIL 4, 2019
1:15 p.m. - 1:55 p.m.

SESSION II: GERM CELL DIFFERENTIATION IN RESPONSE TO LOCAL AND ENDOCRINE FACTORS
Positional Influence on Establishment of the Foundational Spermatogonial Stem Cell Pool
Jon M. Oatley, PhD
Center for Reproductive Biology, School of Molecular Bioscience, College of Veterinary Medicine, Washington State University, Pullman, WA

Continuity, robustness, and regeneration of cell lineages rely on stem cell pools that are established during development. For the mammalian spermatogenic lineage, a foundational pool of spermatogonial stem cells (SSCs) from which all gametes will originate in adulthood arises from prospermatogonial precursors during neonatal life but mechanisms guiding the process are undefined. Using multi-transgenic reporter mouse models, we have mapped the kinetics in vivo, in silico with single-cell RNA sequencing, and functionally with transplantation analyses to define the SSC trajectory from prospermatogonia. Outcomes revealed that SSCs are specified from a subset of quiescent prospermatogonia that are pre-programmed for this fate during fetal development. In recent studies, we have used whole-tissue 3D imaging to explore the topographical arrangement of prospermatogonia and the postnatal spermatogonial subsets that arise from them during neonatal development. We found that prospermatogonia fated to become SSCs are nested in distinct foci throughout seminiferous cords during early neonatal life and expand in number within these nests. After reaching a theoretical number limit as an SSC pool, nest breakdown commences and the cells disperse throughout the tissue mass. In addition, we have uncovered signaling pathways that are activated within clustered germ cells by local signals emanating from somatic cells associated with them that may be key in driving nest establishment and breakdown. Collectively, our findings suggest that positional clustering and intimate interaction with the soma are critical aspects guiding specification of the foundational SSC pool during development. This work was supported by grant HD061665 awarded to JMO from the National Institutes of Health.

THURSDAY, APRIL 4, 2019
1:55 p.m. - 2:35 p.m.

SESSION II: GERM CELL DIFFERENTIATION IN RESPONSE TO LOCAL AND ENDOCRINE FACTORS
Potential Use of Stem Cells for Fertility Preservation
Clifford L. Librach, MC, FRSCS(C), FACOG
Medical and Scientific Director, CReATe Fertility Centre; Professor, University of Toronto, Toronto Canada

Infertility and gonadal dysfunction can result from gonadotoxic therapies, environmental exposures, aging or genetic conditions. Non-obstructive azoospermia (NOA) results from defects in the spermatogenic process that can be attributed to spermatogonial stem cell (SSC) or their niche. While assisted reproductive technologies can enable fertility preservation (FP) in men of reproductive age that are at risk of infertility, FP for pre-pubertal patients remains experimental. Therapeutic options for NOA are limited. The rapid advance of stem cell research and of gene editing technologies could enable new FP options for these patients. Induced pluripotent cells (iPSC), SSC and testicular niche cells, as well as medicinal signaling cells (MSC) have been investigated for their potential use in male FP strategies. We will review the benefits and challenges for three types of stem cell-based approaches under investigation for male FP, focusing on the role that promising sources of MSC derived from human

umbilical cord perivascular cells (HUCPVC) could fulfill. 1. Isolation and ex-vivo expansion of autologous SSC for in vivo transplantation or in vitro spermatogenesis. 2. In vitro spermatogenesis using autologous SSC or adult stem cells such as MSC or iPSC. 3. In vivo protection or regeneration of the spermatogenic niche. We previously reported that first trimester (FTM) and term HUCPVC have increased regenerative properties when compared to older sources of MSC. HUCPVC share many characteristics of testicular MSC in culture, including the expression of extracellular matrix molecules and growth factors known to support the testicular niche. FTM HUCPVC supported the ex-vivo expansion of murine SSC and promoted recovery of spermatogenesis in a phthalate-induced model of testicular damage. FTM and term HUCPVC have the capacity to differentiate towards at least 2 testicular cell lineages in culture, including Sertoli-like cells and haploid germ cell-like cells. Our most recent data suggest that FTM and term HUCPVC are resistant to alkylating drug-induced toxicity in vitro. Intra-testicular delivery of FTM and term HUCPVC prior to treatment with gonadotoxic doses of busulfan led to improved recovery of spermatogenesis and fertility profiles in mice when compared to males receiving injection medium. Our studies suggest that HUCPVC are promising sources of cells that could be utilized in multiple aspects of male FP strategies.

THURSDAY, APRIL 4, 2019
2:55 p.m. - 3:35 p.m.

SESSION II: GERM CELL DIFFERENTIATION IN RESPONSE TO LOCAL AND ENDOCRINE FACTORS

Human Testis Organoid Formation

Ellen Goossens, PhD
Biology of the Testis Lab, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

For nearly a century, researchers have been trying to replicate spermatogenesis in vitro. Two successful 3D culture approaches have been developed in rodents, i.e organ culture and soft agar culture. A key feature of the organ culture system is the preservation of testicular cells in their native 3D configuration: germ cells and Sertoli cells organized in seminiferous tubules and surrounded by interstitial cells. However, this is a limiting factor when manipulation of a particular cell type before culture is desired. In contrast, the soft agar culture does allow single cell input, but hampers easy recovery of cells from the matrix for downstream analyses.

Since these systems were not successful when using human testicular cells, we developed a model that permitted both single cell input and compartmentalization of testicular cells.

In a first attempt, natural cytocompatible scaffolds were derived from human testes. Following recellularization of these scaffolds with primary human testicular cells, testicular organoids were formed. Although testicular organoids were composed of SSCs and their niche cells, testicular organoids lacked the critical testicular organization and, therefore, full differentiation of germ cells was not observed.

In order to better control cell deposition and scaffold design, 3D bioprinting was considered. As 3D bioprinting had never been used to establish spermatogenesis in vitro, two approaches were developed in the mouse model. Testicular constructs consisting of one cell compartment were generated by culturing prepubertal testicular cells in the pores of printed scaffolds. Testicular constructs consisting of two compartments were produced by culturing epithelial cells (CD49f+) in the pores of scaffolds laden with interstitial cells (CD49f-). Spheres were formed in the pores of both the one-compartment and two-compartment construct. Although, restoration of the tubular architecture was not observed, post-meiotic cells including elongated spermatids were found.

FRIDAY, APRIL 5, 2019
8:05 a.m. - 8:55 a.m.

BENCHMARK LECTURE II

Establishing and Replacing Sertoli Cells in the Testis

[1]Blanche Capel, PhD
[1]Tetsuhiro Yokonishi, [1,2]Alexandra Garcia-Moreno, and [1,2]Danielle Maatouk
[1]Duke University Medical Center, Durham, NC
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Sertoli cells are established during gonadal sex determination, the process by which the fetal gonad begins development as either a testis or an ovary. Sex determination depends on a cell fate decision that occurs in the supporting cell lineage of the bipotential gonad, and it is mediated by whether or not Sry, the Y-linked sex-determining gene, and its downstream target Sox9 are expressed. Importantly, upregulation of either Sertoli or granulosa cell pathways is accompanied by mutually antagonistic repressive mechanisms that block the alternate pathway at the time of sex determination, and maintain the cell fate decision throughout adulthood. We found that key sex-determining genes carry bivalent H3K4me3 and H3K27me3 marks prior to sex determination, providing insight into how the bipotential state of gonadal progenitor cells is established. During sex-determination, HeK27me3 is removed at genes involved in Sertoli differentiation, while Cbx2, a component of the polycomb complex, is required to maintain H3K27me3 at genes associated with the female pathway. Our findings support a widespread role for the polycomb complex in repressing the female-determining pathway to establish male fate in supporting cell progenitors.

Efforts to replace Sertoli cells in the adult testis in a clinical setting have been confounded by the inability to deplete the existing Sertoli population safely. By chance, we discovered a common drug that preferentially depletes Sertoli cells in the adult testis, making space for transplantation and establishment of donor Sertoli or donor Sertoli and spermatogonial cells, depending on the timing of transplantation after drug treatment. These novel methods will be valuable for investigation of niche-supporting cell interactions, have the potential to lead to a therapy for idiopathic male infertility in the clinic, and could open the door to production of sperm from other species in the mouse.

This work was supported by a grant to BC from the National Institutes of Health (NICHD R37-HD039963), and a start-up grant to DMM from Northwestern University.

FRIDAY, APRIL 5, 2019
9:00 a.m. - 9:40 a.m.

SESSION III: SOMATIC CELLS FUNCTION IN RESPONSE TO LOCAL FACTORS

Interactions Between Sertoli Cells and Germ Cells Govern the Cycle of the Seminiferous Epithelium

Michael D. Griswold, PhD
Traci Topping, Aileen Helsel and Rachel Gewiss
Center for Reproductive Biology, School of Molecular Biosciences, Washington State University, Pullman, WA 99164

Sertoli cells and germ cells interact throughout testis formation, development, and function in in many complex ways. The two cell types cooperate to constitute many aspects of the structural components of the testis such as intercellular junctions and transport systems. Sertoli cells and germ cells also share biochemical signals that regulate spermatogenic processes such as stem cell and progenitor cell proliferation and the commitment to meiosis. Retinoic acid (RA) is an essential intra- and extracellular signal shared between germ cells and Sertoli cells that affects the onset and maintenance of spermatogenesis. Shortly after birth

in the mouse, RA synthesized by Sertoli cells activates the transition of progenitor undifferentiated spermatogonia to progress into differentiating A1 spermatogonia and initiate the cycle of the seminiferous epithelium in discrete intervals along the tubules. The appearance of A1 spermatogonia triggers a time-regulated differentiation process ultimately ending up in the distinct stages of the cycle and formation of spermatozoa. The cycle is generated by a pulse of RA synthesis from both Sertoli cells and advanced germ cells at appropriate stages of the cycle. Interference with RA synthesis by inhibitors, dietary deficiency, or gene ablation results in a developmental block at the conversion of progenitor cells into differentiating spermatogonia. Exogeneous RA treatment of mice to overcome this interference can lead to the simultaneous progression of progenitor cells into the cycle and spermatogenesis that is synchronous across the entire testis. We have used this synchronization process coupled with cell specific fluorescent markers and cell sorting to examine gene expression in highly purified differentiating spermatogonia and in Sertoli cells associated with different stages of the cycle. The use of the synchronization process to obtain developmentally specific and stage specific germ cells and Sertoli cells will provide new insights into the way these cells interact to produce a functional male gamete. Supported by HD 10808 from NIH.

FRIDAY, APRIL 5, 2019
9:40 a.m. - 10:20 a.m.

SESSION III: SOMATIC CELLS FUNCTION IN RESPONSE TO LOCAL FACTORS

Peritubular Cells of the Human Testis: Prostaglandin E2 (PGE2) and More

Artur Mayerhofer, MD
BMC Munich, LMU, Cell Biology, Anatomy III, D-82152 Planegg-Martinsried, Germany

Several layers of slender, smooth muscle-like cells and extracellular matrix (ECM) form the peritubular compartment of the testis. These myofibroblastic peritubular cells are the least explored testicular cells, but it is thought that they transport sperm. In cases of impaired spermatogenesis, the architecture of the peritubular compartment is altered. ECM deposits and reduced smooth muscle-markers imply that alterations of their functionality occur and are associated with male infertility. While the human testis is not accessible for experimental studies, human testicular peritubular cells (HTPCs) can be isolated from small testicular fragments of patients and then studied in vitro. We have exploited this cellular model, in combination with human testicular samples, to address the question, how peritubular cells contribute to male (in)fertility. We found that HTPCs secrete many factors, which may serve intra-testicular roles, e.g. glial cell line derived neurotrophic factor (GDNF), important for the renewal of spermatogonial stem cells (SSCs). Studies in mutant mice indicated that peritubular cell-derived GDNF is crucial for lifelong spermatogenesis. In mouse peritubular cells androgens enhanced GDNF production, but not in HTPCs. Rather androgen receptors (AR)-activation increased AR and smooth muscle proteins (ACTA2/CNN1) and thus enhanced the smooth-muscle-like phenotype. A proteomic analysis identified the key prostaglandin (PG)-synthesizing enzyme (PTGS1; COX1) and also PGE2-synthesizing enzymes in HTPCs. COX1, the PGE2 receptors EP1, 2 and 4 were found in HTPCs and peritubular cells in situ. Activation of EP1/4 leads to increased GDNF production and CNN1 levels. Ibuprofen is a common drug, which blocks COX1, and it reduced both PGE2 and GNDF production by HTPCs. It also lowered ACTA2/CNN1. If applicable to the in vivo situation, the results suggest that the prototype NSAID ibuprofen may impair important peritubular cell functions and consequently testicular functions. The few examples highlighted, together with several others not mentioned here, indicate that HTPCs provide a useful experimental window into the human testis.
(Supported by DFG)

FRIDAY, APRIL 5, 2019
10:40 a.m. - 11:20 a.m.

SESSION III: SOMATIC CELLS FUNCTION IN RESPONSE TO LOCAL FACTORS

Role of the Interstitial Cells in Spermatogonial Stem Cell Development

Tony De Falco, PhD
Division of Reproductive Sciences, Cincinnati Children's Hospital Medical Center

Spermatogenesis is a complex process which is orchestrated among various cell types within the testis. Interplay between Sertoli and germline lineages within the seminiferous tubule is absolutely critical for sperm differentiation and is well-established in the field. However, interactions between tubular (i.e., Sertoli and germ cells) and interstitial cells, the latter of which are poorly understood, are also vital for fertility. The testicular interstitium contains several cell populations, such as: vascular endothelium; smooth muscle (peritubular and perivascular); pericytes and other perivascular cells; Leydig cells; and testicular macrophages, of which there are at least 2 populations in the adult testis. In particular, the role of macrophages, which can comprise up to 25% of interstitial cells, is unclear in terms of local cellular communication within the testis. Recent findings from our lab have indicated that macrophages are essential for morphogenesis and vascularization of the fetal testis and for differentiation of spermatogonial progenitors in the adult testis. We have found that testicular macrophages have yolk sac origins and undergo significant differentiation between fetal stages and adulthood, likely to create a unique immunosuppressive environment in the mature testis that is conducive to spermatogenesis and to secrete cytokines and growth factors that promote spermatogonial differentiation. These results highlight the wide-ranging roles of immune cells in reproductive biology during various stages of life. Additionally, our studies have uncovered that blood vessels maintain Leydig cell precursors by forming a progenitor niche within the testicular interstitium, which is essential for proper steroidogenesis that is required for sperm formation. Overall, our findings reveal that a diverse population of somatic and immune cells in the testicular interstitium are critical for testicular differentiation, function, and spermatogenesis.

FRIDAY, APRIL 5, 2019
1:15 p.m. - 1:55 p.m.

SESSION IV: TESTICULAR FUNCTION IN RESPONSE TO MAN-MADE CHEMICALS

Effects of Flame Retardants on Testicular Function

Barbara F. Hales, PhD
Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada

Some of the diverse chemicals associated with our modern lifestyle have unintended adverse influences on human health, affecting hormone production or action, and thus acting as endocrine disrupting chemicals (EDCs). Polybrominated diphenyl ether (PBDE) flame retardants are typical EDCs. Animal studies, from our labs and others, have provided evidence for associations between PBDE exposure and adverse outcomes. Treatment of adult male rats with the mixture of brominated

flame retardants found in house dust had an adverse impact on liver drug metabolizing enzymes and thyroid physiology, but not on reproductive organ weights, serum testosterone, testicular function, or sperm DNA integrity. Gestational and lactational BFR exposure altered serum thyroxine in dams and their offspring but did not affect male puberty or parameters of reproduction. However, human studies in Canadian populations demonstrated that every 10-fold increase in maternal hair BDE-99 or 100 was associated with more than a doubling in the risk of having a child with cryptorchidism. In healthy young men from Montreal each 10-fold increase in BDE-47 was associated with lower TSH levels; moreover, BDE-47 exposure was associated with decreased sperm concentration and motility. There is extensive evidence that PBDEs also have adverse health effects on neurodevelopment and female reproduction. Together, these data have led to worldwide government restrictions of their use, creating a "market gap" and the emergence of organophosphate esters (OPEs) as replacement flame retardants. Using in in vitro cell cultures we have shown that some of these chemicals are as, or more, toxic than the PBDEs they have replaced. A significantly reduction in mitochondrial activity was observed in MA-10 Leydig cells exposed to BDE-47 and all 7 of the OPEs tested. Basal progesterone production was significantly increased by 5 of the OPEs; BDE-47 and 2 others had no effect. It appears essential that we improve how we assess replacements, from exposure to biological effects, to ensure that strategies are in place to identify alternative chemicals that are safer and have a reduced environmental impact. Supported by CIHR team grants. BFH is a James McGill Professor.

FRIDAY, APRIL 5, 2019
1:55 p.m. - 2:35 p.m.

SESSION IV: TESTICULAR FUNCTION IN RESPONSE TO MAN-MADE CHEMICALS

Phthalate Exposure on Sperm Epigenetics and Early-life Development in Man and Mouse

J. Richard Pilsner, PhD, MPH
University of Massachusetts Amherst

Introduction: Phthalates, a class of endocrine disrupting compounds (EDCs) used in plastics and personal care products, are ubiquitous environmental contaminants resulting in widespread human exposure. In humans, male phthalate exposure is associated with poor sperm quality and longer time to pregnancy. An emerging body of data suggests that environmental exposures can be embodied within the developing male germ cell as epigenetic marks, and in turn, affect the trajectory of offspring health and development. We seek to examine the associations between preconception phthalate exposure and sperm DNA methylation in mice and man.
Methods: Men from the first 48 couples from the Sperm Environmental Epigenetics and Development Study (SEEDS) provided a spot urine sample for phthalate measurements on the same day as semen sample procurement. Sperm DNA methylation was assessed with the HumanMethylation 450K array. For mouse studies, eight-week old C57BL/6J male mice were exposed to either a vehicle control, a low dose of DEPH (2.5mg/kg/weight), or a high dose of DEPH (25mg/kg/weight) for 70 days. Following the exposure, males were mated with unexposed, 8-12-week old, DBA/2J females.
Results: Adjusting for age, BMI, and current smoking, 131 DMRs were associated with at least one urinary metabolite. Most sperm DMRs were associated with anti-androgenic metabolites, including MEHP (n=83), MEOHP (n=16), MBP (n=22), and MCOCH (n=7). Functional analyses of DMRs revealed enrichment of genes related to growth and development as well as cellular function and maintenance. Finally, 13% of sperm DMRs were inversely associated with high quality blastocyst-stage embryos after IVF. In mice embryonic and extra-embryonic lineagese, paternal DEPH exposure resulted in differential expression of genes related to important developmental processes, including tissue morphogenesis, blood vessel development, placenta development, and cell fate commitments.
Conclusions: These results suggest that preconception environmental conditions in males influence early-life development likely via epigenetic reprogramming of sperm during spermatogenesis.

FRIDAY, APRIL 5, 2019
2:55 p.m. - 3:35 p.m.

SESSION IV: TESTICULAR FUNCTION IN RESPONSE TO MAN-MADE CHEMICALS

Effects of Endocrine Disruptor Mixtures on Male Reproduction

Martine Culty, PhD
Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA USA.

Humans are simultaneously exposed to mixtures of chemicals of anthropogenic and natural origins, depending of their living environment, lifestyle and dietary habits. Some of these chemicals are known to act as endocrine disruptors in wild life and laboratory animals. Particularly, they exert adverse reproductive effects by disrupting androgen and estrogen homeostasis during critical windows of perinatal development. This is the case of the antiandrogenic phthalate plasticizers and the fungicide vinclosolin, as well as the xenoestrogen bisphenol A and the phytoestrogen genistein. Recent epidemiological studies indicated strong association between exposure and endocrine disruption in humans for some of these compounds. Our goal was to go beyond the single chemical-based risk assessment approach, traditionally used in regulatory toxicology, by examining the effects of a prototype binary mixture of two such chemicals, at doses relevant to human exposure. Di-(ethylhexyl) phthalate and genistein were selected as a conceivable mixture, since they are detected both in infants and adults. Pregnant Sprague Dawley rats were exposed to vehicle or the two compounds, alone or mixed, from gestation day 14 to birth. The reproductive system of the male F1 offspring, as well as their F2 and F3 descendants were examined, revealing long-term and multigenerational adverse effects, more pronounced for the mixture. Gene expression analysis identified novel target genes and functional pathways, as well as the contribution of several testicular cell types in these effects. These studies support including mixtures in the process of chemical risk assessment.

SATURDAY, APRIL 6, 2019
8:05 a.m. - 8:55 a.m.

BENCHMARK LECTURE III

Testis & Plastics: 25+ Years of the Estrogen Hypothesis

Kim Boekelheide, MD, PhD
Brown University

Over 25 years ago, Sharpe and Skakkebaek proposed the estrogen hypothesis to explain the complex male reproductive effects observed in human and animal studies of diethylstilbestrol and other estrogenic endocrine disrupting chemical exposures. The estrogen hypothesis subsequently expanded and evolved into the Testicular Dysgenesis Syndrome (TDS) to account for temporal trends in sperm counts (↓), hypospadias (↑), cryptorchidism (↑), and testis germ cell cancer (↑). And then……plastics!
Phthalates are ubiquitous plasticizers, and produce the hallmarks of TDS following exposure of male rat fetuses, the eponymous Phthalate Syndrome. In the rat, phthalates act as potent anti-androgens, inhibiting

steroidogenesis in the fetal testis during critical stages of male reproductive tract development. While phthalates are not estrogenic, another ubiquitous plasticizer, bisphenol A (BPA), is estrogenic. Numerous Good Laboratory Practices (GLP) and academic studies have produced conflicting results regarding the potential adverse effects of BPA exposure.

The resulting uncertainty and widespread exposure to BPA led the Food and Drug Administration and the National Institute of Environmental Health Sciences to support a large research effort combining a core GLP study conducted at the National Center for Toxicological Research (NCTR) with investigations in a dozen academic laboratories, the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA). The NCTR GLP study provided our laboratory with rat testes, epididymides, and sperm collected at 90 days of age after continuous exposure from early gestation to a wide range of BPA doses. Effects of BPA on body, testis and epididymis weights were seen only at the highest administered dose of BPA (250,000 µg/kg/d). Transcriptomic and epigenomic analyses of sperm mRNA and DNA methylation did not identify reproducible alterations associated with BPA exposure. These data indicate that near lifetime exposure to BPA over a wide range of doses has little impact on the endpoints evaluated for testes, epididymides, or sperm molecular profiles of 90 day old rats.

SATURDAY, APRIL 6, 2019
9:00 a.m. - 9:40 a.m.

SESSION V: PATHOGEN EFFECTS ON TESTIS FUNCTION

The Impact of ZIKA Infection on Male Fertility and the Role of Vaccination

Gary Kobinger, PhD, [1,2,6,7]
Marc-Antoine de La Vega[1,2], Raquel das Neves Almeida[3], Kar Muthumani[4], Kelly G. Magalhães3, Guy Boivin[2,3], Joel N Maslow[5], David B Weiner[4]
[1]Département de Microbiologie-Infectiologie et d'Immunologie, Université Laval, Québec, Canada, [2]Centre de Recherche en Infectiologie du CHU de Québec - Université Laval, Canada, [3]Laboratory of Immunology and Inflammation, University of Brasilia, Brazil, [4]Vaccine & Immunotherapy Center, The Wistar Institute, Philadelphia, Pennsylvania, [5]GeneOne Life Science Inc., Seoul, Korea, [6]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada, [7]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia

Considered a Public Health Emergency of International Concern in 2016, Zika virus (ZIKV), an emerging arbovirus of the Flaviviridae family, caused outbreaks in Africa, Asia, the South Pacific and the Americas over the past few decades. Several congenital abnormalities and malformations can be associated with ZIKV infection, as well as a high number of neurological diseases in adults. Involvement of the male reproductive tract in association with ZIKV infection has been reported. However, the mechanisms and relations of viral infection along with the male reproductive effects remain unclear. ZIKV was first described as being transmitted by mosquitoes, however sexual transmission also occurs. In an attempt to prevent or stop the spread of ZIKV, vaccine development was accelerated through substantial global scientific efforts. Candidate vaccines have shown high protective efficacy in different models. A consensus DNA vaccine (GLS-5700) encoding the ZIKV pre-membrane and envelope proteins was shown to protect IFNAR-/- mice against ZIKV-induced damage to testicles and spermatozoa. Interestingly, vaccination was found to also prevent ZIKV persistence in the male reproductive tract. While ZIKV-induced male infertility can sometimes revert naturally, the molecular determinants of successful reversion remain unknown. More studies are necessary to highlight the mechanisms associated to testicular protection by vaccination as well as the mechanism of reversion. Our data indicate that ZIKV can abrogate male fertility temporarily or possibly permanently, which is preventable through immunization.

SATURDAY, APRIL 6, 2019
10:00 a.m. - 10:40 a.m.

SESSION V: PATHOGEN EFFECTS ON TESTIS FUNCTION

Newly Emerging Sexually Transmitted Viruses: Testicular Defense Systems and Clinical Implications

Saguna Verma, PhD
Associate Professor, Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI.

The spectrum of pathogens that can be sexually transmitted is broad and now includes Zika (ZIKV), Ebola and Lassa viruses as the recently recognized sexually transmissible pathogens. ZIKV is shown to be present in human seminal fluid for up to six months after infection and associated with low sperm count. Similarly, sexual transmission of Ebola virus is documented and sheer number of male survivors from recent outbreaks raises concern about potential flare-ups in new geographical regions and generation of new virus strains. Epidemiological data collectively indicate the ability of these viruses to hide in the immune privilege compartment of the seminiferous tubules to escape peripheral immunity. The human testes immune environment is immunosuppressive in nature and is tightly governed by an elaborate communication network between different cell types including Sertoli cells (SC) that produce cytokines and hormones required for maintaining local immune homeostasis. Our group focuses on understanding immune mechanisms of ZIKV persistence in the testis. We demonstrated that primary human SC were highly susceptible to ZIKV as compared to other cell types. Using an in vitro human blood-testes barrier model we further showed that ZIKV transmigrated across the barrier to the luminal side. Subsequent RNA-seq analysis revealed modulation of pathways associated with antiviral defense and SC-germ cells network, thus suggesting that ZIKV may affect the function of the seminiferous epithelium. Our preliminary study also provides evidence that multicellular human testicular organoids can be used as an ideal tool to study ZIKV immunopathogenesis and effect on germ cells. The ability of ZIKV to establish persistent infection only in the testes clearly suggests involvement of unique immune suppressive mechanisms. Ongoing studies in this direction indicate that AXL receptor signaling plays an important role in not only facilitating robust ZIKV entry into the SC but also negatively modulates antiviral defense pathways. Our data and newly developed tools lay the groundwork for future translational studies including testing efficacy of antiviral drugs to clear testicular infection of ZIKV and other emerging testicular pathogens.

SATURDAY, APRIL 6, 2019
10:40 a.m. - 11:20 a.m.

SESSION V: PATHOGEN EFFECTS ON TESTIS FUNCTION

The Immunology of Male Reproduction

Mark Hedger, PhD
Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, Australia

Spermatogenic cells are particularly susceptible to damage by the immune system, due to the absence of tolerance for many antigens associated with spermatogenesis. This presents a conundrum for male reproduction. On the one hand, male gametes must be protected from immunological damage, but, on the other hand, any deficiency in the

immune response would render the male reproductive tract less capable of resisting infection and tumours. The testis is susceptible to various viral infections, while the epididymis and vas deferens are targets for ascending bacterial infections, and it is clear from animal models and the clinic that immunological responses to these infections can lead to both acute and chronic inflammation, genital duct damage, sperm autoimmunity, infertility and pain.

It is now well-established that the spermatogenic cells in the testis are protected by specialised immunoregulatory and immunosuppressive mechanisms. The somatic cells of the testis, and the Sertoli cells in particular, are primarily responsible for regulating this protection, although many aspects of the process are still to be discovered. Crucially, under the influence of the testicular environment, resident macrophages and dendritic cells play a key role in immunoregulation through their ability to control antigen-specific activation, but these immune cells are also increasingly implicated in testis development and the regulation of spermatogenesis and steroidogenesis.

Immunological events in the epididymis and vas deferens, where sperm mature and are stored prior to ejaculation, can also have detrimental effects on spermatogenesis and fertility. The immune environments of the epididymis and vas display significant differences to that of the testis,. However, in both the testis and the epididymis, cytokines of the transforming growth factor-β family, most notably the activins, are implicated in this regulation.

Elucidation of the unique interface between the male reproductive tract and the immune system is an essential factor in the understanding and treatment of most male reproductive health pathologies.

SHORT TALK ABSTRACTS

Short Talk #1

SINGLE-CELL TRANSCRIPTOMES OF PROSPERMATOGONIA REVEAL HETEROGENEITY IN THE RA RESPONSE IN THE NEONATAL MOUSE TESTIS

Anukriti Singh Master of Science[1], Ellen K. Velte PHD[2], Christopher B. Geyer PHD[3] and Brian P. Hermann PHD[1]
[1]Department of Biology, University of Texas at San Antonio; [2]Departments of Anatomy and Cell Biology, Brody School of Medicine, East Carolina University, Greenville, NC, USA; [3]East Carolina Diabetes and Obesity Institute, East Carolina University, Greenville, NC, USA
(Presented By: Anukriti Singh, B.Tech)

Mammalian spermatogenesis begins with the segregation of prospermatogonia into type A undifferentiating and differentiating spermatogonia by postnatal days 3-4 (P3-4), which temporally coincides with the onset of retinoic acid (RA) signaling. Spermatogonial stem cells (SSCs) are a subset of undifferentiated spermatogonia that sustain spermatogenesis through the balanced production of progeny that will either retain stemness (self-renew) or initiate the process of differentiation by acquiring the ability to respond to RA. The mechanisms underlying differential spermatogonial competency for RA response are unknown. Using both in vitro and in vivo pharmacological approaches, we previously determined that CYP26-mediated RA catabolism prevents the majority of neonatal prospermatogonia from responding to RA. However, a subset of prospermatogonia representing putative SSC precursors are RA-insensitive and protected via a CYP26-independent mechanism. To explore differential RA responsiveness, we performed single-cell RNA-Seq on 34,153 testis cells from P1.5 mice treated with RA or DMSO. This analysis confirmed that only a subpopulation of prospermatogonia responded to RA by inducing known RA-regulated genes (e.g., Cpm, Cypb26a1, Cyp26b1, Rarb, Stra8). RA-responsive prospermatogonia (n=247) (Stra8+ or Sod3+ or Rarb+) exhibited elevated expression levels of 27 genes involved in G2/M DNA Damage checkpoint regulation (Cks1b, Top2a, Ccnb1), GADD45 Signaling (Ccnd2, Ccnb1) and Wnt/β-catenin signaling (Sox4, Rarb, Ubc). Only 3 genes were elevated in prospermatogonia (n=125) that did not exhibit an RA response and they were involved in EIF2 signaling (Rpl6). Collectively, these findings reveal multiple mechanisms, such as CYP26B1-mediated catabolism and absence of RA receptor expression, that protects subpopulations of prospermatogonia, including putative SSCs, from RA-induced differentiation.

Short Talk #2

DIRECTING HUMAN INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATION TOWARDS LEYDIG AND ADRENAL CELLS

Lu Li PhD, Yuchang Li PhD, Chantal Sottas BSc, Martine Culty PhD and Vassilios Papadopoulos DPharm, PhD
Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA
(Presented By: Lu Li, PhD)

Reduced serum testosterone (T), or hypogonadism, affects millions of men. Hypogonadism has been found to be associated with conditions that include infertility, cardiovascular diseases, altered mood, fatigue, decreased lean body mass, reduced bone mineral density, increased visceral fat, metabolic syndrome, and decreased libido and sexual function. Reduced serum T is common in aging men where Leydig cells are becoming less responsive to LH referred to as primary hypogonadism, a condition that also occurs in 50% of men diagnosed with idiopathic infertility. Administering T-replacement therapy (TRT) reverses many of the symptoms of low T levels. However, TRT is linked to side effects such as infertility, and increased risk of prostate cancer and cardiovascular disease. Thus, there is a need to obtain T-producing cells, which could be used to treat hypogonadism based on transplantation and reestablishment of T-producing cell lineage in the body. In humans, T is synthesized by Leydig cells (LCs) likely deriving from mesenchymal cells of mesonephric origin. Although mesenchymal cells have been successfully induced into LCs, the limited source and possible trauma to donors hinders their use in clinical therapies. Alternatively, human induced pluripotent stem cells (hiPSCs) that are highly expandable in cell culture and have the potential to differentiate into all somatic cell types become the emerging source of autologous cell therapies. We have successfully induced the differentiation of hiPSCs through mesoderm and mesenchymal cell progenitors into either human Leydig-like cells (hLLCs) or human adrenal-like cells (hALCs) under different culture conditions. Factors critical for the normal development of LCs were added to both culture systems. hLLCs (i) express all genes specific for Leydig cells and important for T biosynthesis, (ii) synthesize T, rather than cortisol (F), in response to dibutyryl-cAMP and 22R-hydroxycholesterol, and (iii) display ultrastructural features resembling LCs. In contrast, hALCs (i) express steroidogenic genes contributing to cortisol (F) biosynthesis and (ii) synthesize F rather than T in response to the same stimuli. These data suggest that under appropriate culture conditions hiPSCs can be driven to form either LCs or ACs. This bidirectional approach offers an insight into the differentiation of steroidogenic cells originating from the same fetal precursors and unveils new venues for cell therapy to treat hypogonadism.

Short Talk #3

SINGLE-CELL RNA SEQUENCING REVEALS NOVEL MARKERS OF STEM/PROGENITOR SPERMATOGONIA IN HIGHER PRIMATES

Sarah Munyoki BA[1], Adrienne Shami BS[2], Qianyi Ma PhD[2], Chris Green PhD[2], Jun Li PhD[2], Sue Hammoud PhD[2] and Kyle Orwig PhD[1]
[1]University of Pittsburgh; [2]University of Michigan
(Presented By: Sarah Munyoki, BA)

In the adult male testis, spermatogonial stem cells (SSCs) are essential for continuous spermatogenesis to maintain fertility throughout life. SSCs exquisitely balance self-renewal and differentiation through molecular mechanisms that are still poorly understood, especially in higher primates. SSC transplantation as a therapy for male infertility, is well established in rodents and may have application in the human clinic. However, while many features of testicular biology are conserved from rodents to higher primates, there is divergence in stem cell phenotype and spermatogenetic lineage development. Determining the unique features of higher primate SSCs will facilitate the translation of SSC based therapies to the human fertility clinic. We employed Drop-Seq for high throughput, unbiased, single-cell RNA-sequencing of healthy adult higher primates (human and rhesus macaque) testicular tissue, generating ~33,800 single cell transcriptomes. Dimensionality reduction and unsupervised clustering methods partitioned the cells into transcriptionally distinct populations, representing all known and potentially novel cell types of higher primate testes. Further analysis of our data has identified novel genes GPX1, MORC1, GPC4 and GPC3 as potential markers of human and monkey stem/progenitor spermatogonia. These genes are known to have diverse cellular functions and are involved in various signaling pathways such as WNT, Hedgehog, FGF, BMP, MAP2K/AKT and degradation of reactive oxygen species that may be important in regulating higher primate SSC function. Our single cell data may reveal novel mechanisms regulating higher primate SSCs that can be exploited for sorting, enhancing survival and expansion in culture or other applications that improve the fundamental knowledge about SSCs in higher primates, and may enable applications in the male infertility clinic.

SHORT TALK ABSTRACTS

Short Talk #4

DYNAMIC SUBCELLULAR MEMBRANE LIPID REMODELING IN HORMONE-INDUCED LEYDIG CELL STEROIDOGENESIS

Sathvika Venugopal PhD[1], Rachel Chan BSc[1], Esha Sanyal BSc[1], Lorne Taylor MSc[1], Pushwinder Kaur MSc[1], Edward Daly BSc[1] and Vassilios Papadopoulos DPharm, PhD, DSc [2]

[1]Research Institute of the McGill University Health Centre, Montreal, Quebec, H4A 3J1, Canada; [2]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA

(Presented By: Sathvika Venugopal, PhD)

Cholesterol is the sole precursor for all steroids produced in mammals. During acute steroidogenesis, large quantities of cholesterol are trafficked from its intracellular stores to the cholesterol-poor organelle, mitochondria, to be converted to pregnenolone. Although steroidogenesis is extensively studied, the actual source organelle that stores and then mobilizes cholesterol for steroid production and the pathway that assists in trafficking this hydrophobic molecule to mitochondria are yet to be determined. Utilizing the domain 4 (D4) of the Perfringolysin O protein produced by Clostridium perfringens, which binds to high concentrations of cholesterol in membranes without cytotoxicity, we first determined the source organelle. Live cell imaging analysis in a Leydig tumor cell line and primary rat Leydig cells utilizing mCherry tagged D4 revealed release of a pool of cholesterol from the plasma membrane within 30 minutes of hormonal stimulation. These observations led us to conclude that the bulk of steroidogenic cholesterol destined for mitochondria originates from the plasma membrane during acute steroidogenesis. We further identified a pregnenolone-mediated feedback mechanism that stops excessive cholesterol movement from the plasma membrane and thus protects mitochondria from cholesterol-induced toxicity. A precise mechanism that facilitates rapid and sustainable movement of large quantities of cholesterol to the mitochondria had yet to be determined. Previous studies showing an increased interaction between the endoplasmic reticulum and mitochondria during acute steroidogenesis, led us to hypothesize that cholesterol from the plasma membrane enters the endoplasmic reticulum via a membrane association and thus reaches mitochondria by plasma membrane – endoplasmic reticulum – mitochondria associations called PAMs. These membrane associations and cellular signals are facilitated by a variety of lipid classes. Hence, we addressed this hypothesis by subcellular fractionation of hormonally induced and also hormonally induced but steroidogenesis-inhibited MA-10 cells subjected to lipidomics analysis utilizing mass spectrometry. The results obtained from this study support the notion that PAMs are the route for cholesterol movement from plasma membrane to the mitochondria. Further, we also noted a dynamic reorganization of multiple lipid classes that facilitate the membrane associations and cellular signals.

Short Talk #5

THE ROLE OF RNA BINDING PROTEIN ADAD2 IN MALE MEIOSIS

Lauren Chukrallah, Gabriella Acoury and Elizabeth Snyder PhD

Rutgers the State University of New Jersey

(Presented By: Lauren Chukrallah)

Meiosis, the specialized cell division responsible for genetic recombination, is an essential, germ-cell-specific process. Successful meiosis is necessary for fertility and requires a unique set of molecular mechanisms including special RNA biogenesis events. Many of these events are mediated by meiosis-specific RNA binding proteins (RBPs). One such RBP, adenosine deaminase domain containing protein 2 (ADAD2), is expressed predominantly in meiotic spermatocytes and displays a developmentally regulated pattern of expression. Immunofluorescence in adult mouse testis showed ADAD2 is diffusely cytoplasmic in early pachytene and then forms perinuclear foci by late pachytene. To interrogate the function of ADAD2, a global Adad2 knockout mouse allele was produced via CRISPR-Cas9 mutagenesis of the endogenous Adad2 locus. This created a single nucleotide deletion which resulted in a frameshift, abrogating protein production. Adad2 knockout males are infertile with significant loss of post-meiotic germ cells. Given its meiotic expression and early post-meiotic germ cell defects in the mutant, the impact of ADAD2 loss on meiosis was assessed. Histological evaluation of Adad2 knockout testes revealed a trend toward decreased MII spermatocytes. To more accurately assess meiotic progression, immunocytochemical analyses of meiotic cell spreads from ADAD2 knockout mice were performed. These analyses demonstrated increased frequency of early pachytene spermatocytes and decreased frequencies of late pachytene and diplotene spermatocytes. These data indicate that ADAD2 is required for optimal meiotic progression. A this defect coincides with a key pachytene progression checkpoint future work will focus on quantifying markers of the pachytene checkpoint failure to investigate whether the checkpoint is being triggered in Adad2 knockout spermatocytes.

Short Talk #6

UNRAVELLING THE ROLE OF TRIM28 IN SPERMATOGENESIS

Joel Tan PhD and Daniel Messerschmidt PhD

IMCB, A*STAR

(Presented By: Joel Heng Loong Tan, BSc (Hons)

The role of epigenetics in spermatogenesis is becoming more apparent and male infertility is increasingly linked back to epigenetic defects. Tripartite motif-containing 28 (TRIM28) is an indispensible epigenetic transcriptional co-regulator that has been shown to regulate numerous biological processes including cellular differentiation. While little is understood about the role of TRIM28 in spermatogenesis, deleting it leads to testicular degeneration in mice, which warrants further investigation. In our laboratory, we observed that Trim28-heterozygous (Trim28Het) male mice became infertile prematurely, pointing to a likely haploinsufficiency phenotype of Trim28. Mating experiments confirmed this observation. As these mice grew older, their testes progressively shrank compared to the wild type. Histological analysis uncovered an increase in Sertoli cell-only tubules, suggesting that the size reduction could be attributed to the loss of germ cells. Since the degenerative phenotype might be a consequence of systemic Trim28-heterozygosity, we generated and compared germ cell-specific and Sertoli cell-specific heterozygotes. We found that halving the amount of TRIM28 in the germ cells phenocopied Trim28Het testes, indicating a germ cell autonomous effect. Survey of the germ cell population in the seminiferous tubules revealed that heterozygous germ cells are progressively lost, beginning with the undifferentiated spermatogonia. Contrary to previous publication, which showed that TRIM28 was only expressed in mid-pachytene spermatocytes to early elongating spermatids, we detected Trim28 expression in undifferentiated spermatogonia. Based on these results, TRIM28 appears to have a function in these early cells of spermatogenesis where the stem cell population resides. Preliminary results suggest that TRIM28 regulates the fate of the spermatogonial stem cells.

SHORT TALK ABSTRACTS

Short Talk #7

FUNCTIONAL ROLE OF THE COPPER TRANSPORTER 1 PROTEIN IN SPERMATOGENESIS AND IN CISPLATIN-INDUCED TESTICULAR INJURY

Rashin Ghaffari[1], Kristin Di Bona PhD[2] and John Richburg PhD[2]
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(Presented By: Rashin Ghaffari, BS)

Cisplatin (cDDP) is a highly effective chemotherapeutic drug. However, treatment with cDDP contributes to many adverse side effects including prolonged azoospermia in male patients. Both acute and sub-chronic exposure to cDDP induces testicular germ cell (GC) loss and prolonged azoospermia, respectively, in animal models. Although it has been well documented that cDDP exposure induces male reproductive toxicity and disruption to spermatogenesis, the mechanism (s) that account for the prolonged infertility as a result of cDDP treatment is not understood. The high affinity membrane copper transporter 1 (CTR1; SCL31A1) protein, which functions as a copper (Cu) importer in mammalian cells, has been shown to be highly associated with cDDP sensitivity and accumulation in mammalian cells. Our preliminary evaluation on mice testis indicates that CTR1 is primarily expressed in primary spermatocytes and SCs. To examine the role of CTR1 in cDDP-induced testicular injury, we have developed two independent mouse models, with the conditional knockout of Ctr1 in either SCs (SC-KO; Amh-Cre, Ctr1fl/Δ) or GCs (GC-KO; Ddx4-Cre, Ctr1fl/Δ). Interestingly, GC-KOs exhibit a severe reduction in testis weight (~83% by PND 41) with complete depletion of post-meiotic GCs. On the other hand, SC-KO mice were fertile and had indistinguishable testis weight and histology from their wild-type (WT; Ctr1fl/fl) littermates. The SC-KO mice were further challenged with an acute dose of cDDP, where the SC-KO and WT mice were either exposed to a single high dose of 5 mg/kg of cDDP or equivalent volume of saline for 48 hours. We found that while cDDP treated WT mice had twofold increase in GC death compared to saline treated WT mice, cDDP treated SC-KO mice exhibited only half fold increase in GC death compared to saline treated SC-KO mice. Moreover, platinum levels in cDDP treated SC-KO mice testis were significantly lower than in WT mice testis. Taken together, these observations reveal for the first time 1) the essential role of CTR1 in GCs, but not in SCs, for functional spermatogenesis and, 2) the functional significance of CTR1 expressed by SCs on mediating cDDP-induced GC loss. Future investigations will utilize SC-KO as a mouse model to study the contribution of the environmental niche provided by the SC on spermatogenesis followed by clinically relevant multi-dose cDDP treatment, and the GC-KO mice will be utilized to explore the importance of CTR1 and/or Cu on spermatogenesis.

Short Talk #8

HIPK4 IS ESSENTIAL FOR MURINE SPERMIOGENESIS

Aaron Crapster PhD[1], Paul Rack PhD[2], Zane Hellmann BA[1], Barry Behr PhD[1], Yanfeng Li[1], Jennifer Lin MS[1], Hong Zeng MD, PhD[1] and James Chen PhD[1]
[1]Stanford University; [2]Thermo Fisher Scientific
(Presented By: J. Aaron Crapster, PhD)

Introduction: Spermiogenesis is a remarkable cellular transformation in which haploid round spermatids become elongated spermatozoa capable of motility and fertilization. Previous studies have revealed that many cytological changes are associated with this process, including formation of the actin-scaffolded acrosome/acroplaxome complex, nuclear reshaping, and flagellum assembly. However, the signaling pathways that coordinate these steps remain enigmatic. Here we demonstrate that an atypical member of the homeodomain-interacting protein kinase family, HIPK4, is essential for spermiogenesis and male fertility.
Objectives: Our goal was to characterize the reproductive phenotypes associated with the loss of HIPK4. Methods. Hipk4 expression was characterized by qPCR, in situ hybridization, and immunofluorescence studies. Hipk4 knockout mice were tested for their fertilization potential by mating, in vitro fertilization, and intracytoplasmic sperm injection (ICSI). Hipk4 mutant sperm were further evaluated for their ability to capacitate, undergo the acrosome reaction, and bind to oocytes. Electron microscopy and immunofluorescence studies were conducted on testis sections, isolated spermatids, and epididymal sperm. RNA profiling of Hipk4 knockout testes was performed by microarray analysis. HIPK4-dependent phosphorylation events in cultured fibroblast cells were identified by quantitative mass spectrometry.
Results obtained: HIPK4 is expressed in round and elongating spermatids. Male Hipk4 knockout mice are infertile, exhibiting oligoasthenoteratozoospermia, with no other overt physiological defects. HIPK4-deficient sperm exhibit diminished oocyte binding and are incompetent for in vitro fertilization, but produce viable pups via ICSI. Ultrastructural analyses reveal subtle acrosome/acroplaxome defects that arise in elongating Hipk4 mutant spermatids and lead to abnormal head morphology. Loss of Hipk4 does not dramatically alter testis-wide transcript expression; however, HIPK4 overexpression alters actin and Golgi dynamics in cultured somatic cells, and modulates the phosphorylation of known actin regulators.
Conclusions: Our findings establish HIPK4 as a critical regulator of spermatid head shaping and a potential contraceptive target.

POSTER SESSIONS

Poster #1
WITHDRAWN

Poster #2
THE MOUSE STRAIN MATTERS: SUCCESS OF IN VITRO SPERMATOGENESIS IS DEPENDENT ON GENETIC BACKGROUND OF DONOR MICE
Joana M.D. Portela MSc[1], Callista L. Mulder PhD[1], Saskia K.M. van Daalen BSc[1], Cindy M. de Winter-Korver BSc[1], Sjoerd Repping Prof Dr (PhD)[1], Jan-Bernd Stukenborg Assoc Prof (PhD)[2] and Ans M.M. van Pelt Prof Dr (PhD)[1]
[1]Amsterdam UMC, University of Amsterdam, Center for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.; [2]Karolinska Institutet and University Hospital, Paediatric Endocrinology Unit, NORDFERTIL Research Lab Stockholm, Department of Women's and Children's Health, Visionsgatan 4, J9:30, , SE-171 64 Solna, Sweden.
(Presented By: Callista L. Mulder, PhD)

Poster #3
EXPRESSION OF THE GLUCOCORTICOID RECEPTOR NR3C1 IN TESTICULAR PERITUBULAR CELLS IS DEVELOPMENTALLY REGULATED AND LINKED TO THE SMOOTH MUSCLE-LIKE PHENOTYPE, ELASTIN AND CYTOKINE PRODUCTION
Harald Welter, Nils Dellweg, Carola Herrmann, Kim Dietrich, Bastian Popper, Henryk Urbanski, J. Ullrich Schwarzer, Frank-Michael Köhn and Artur Mayerhofer
(Presented By: Artur Mayerhofer, MD)

Poster #4
DIRECTING HUMAN INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATION TOWARDS LEYDIG AND ADRENAL CELLS
Lu Li PhD, Yuchang Li PhD, Chantal Sottas BSc, Martine Culty PhD and Vassilios Papadopoulos DPharm, PhD
Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA
(Presented By: Lu Li, PhD)

Poster #5
MITOCHONDRIAL TSPO DEFICIENCY TRIGGERS RETROGRADE SIGNALING THROUGH DYSREGULATED CYTOSOLIC CALCIUM HOMEOSTASIS IN MA-10 MOUSE TUMOR LEYDIG CELLS
Jinjiang Fan PhD[1] and Vassilios Papadopoulos DPharm, PhD, DSc (hon)[2]
[1]The Research Institute of the McGill University Health Centre and Department of Medicine, McGill University, Montreal, Quebec, Canada; [2]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California
(Presented By: Vassilios Papadopoulos, PhD, DSc (hon))

Poster #6
INTRAFLAGELLAR TRANSPORTER PROTEIN 27 (IFT27) IS ESSENTIAL FOR TRANSPORTING SIGNAL PEPTIDE PEPTIDASE-LIKE 2A (SPPL2A) TO PROCESS PRECURSOR A-KINASE ANCHOR PROTEIN 4 (PRO-AKAP4) TO MATURE AKAP4 FOR ASSEMBLY OF FUNCTIONAL SPERM
Qian Huang, Shiyang Zhang, Bernd Schröder and Zhibing Zhang
(Presented By: Qian Huang)

Poster #7
TESTICULAR MACROPHAGES IN ANDROGEN INSENSITIVITY SYNDROME
Paula Aliberti Doctoral Student[1], Marco A Rivarola MD PhD[1], Alicia Belgorosky MD PhD[2] and Esperanza Berensztein PhD[1]
[1]Garrahan Pediatric Hospital; [2]Research Institute Garrahan Conicet
(Presented By: Esperanza Berensztein, PhD)

Poster #8
CRISPR/CAS9-MEDIATED STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR) KNOCKOUT IN MA-10 CELLS PROVIDES INSIGHT INTO RELATIONSHIP BETWEEN STAR AND TRANSLOCATOR PROTEIN TSPO
Melanie Galano[1], Yasaman Aghazadeh PhD[2], Yuchang Li PhD[1] and Vassilios Papadopoulos DPharm, PhD, DSc (Hon)[1,2]
[1]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA; [2]Research Institute of McGill University Health Center and Department of Medicine, McGill University, Montreal, Quebec, Canada
(Presented By: Melanie Galano)

Poster #9
INFLUENCE OF ETHANOL CONSUMPTION ON THE CELL STRUCTURE OF SPERMATOGENIC EPITHELIUM AND SPERM
Anastasiya Spaska MSc, PhD[1] and Neonila Dolynko PhD[2]
[1]Girne American University, Turkey; [2]Precarpathian National University, Ukraine
(Presented By: Anastasiya Spaska, MSc, PhD)

Poster #10
DYNAMIC SUBCELLULAR MEMBRANE LIPID REMODELING IN HORMONE-INDUCED LEYDIG CELL STEROIDOGENESIS
Sathvika Venugopal PhD[1], Rachel Chan BSc[1], Esha Sanyal BSc[1], Lorne Taylor MSc[1], Pushwinder Kaur MSc[1], Edward Daly BSc[1] and Vassilios Papadopoulos DPharm, PhD, DSc (Hon)[2]
[1]Research Institute of the McGill University Health Centre, Montreal, Quebec, H4A 3J1, Canada; [2]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA
(Presented By: Sathvika Venugopal, PhD)

Poster #11
WHAT IS THE BETTER CULTURE MEDIA FOR IMPROVE THE MOTILITY OF ASTHENOZOOSPERMIC SAMPLES IN ANAEROBIC CONDITIONS?
Caroline Ranéa BSc, MSc student[1,2,3], Juliana Risso Pariz PD fellow[1,4,5,6], Rosa Alice Casemiro Monteiro BSc[7], Bruna Zillig BSc student[7], Heloisa Faquineti BSc, MSc student[7], Donald Evenson PhD[8], Joel Drevet PhD[7], Giovanna Milani MD student[7] and Jorge Hallak MD, PhD[1,4,5,6]
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(Presented By: Caroline Ranéa, BSc, MSc student)

POSTER SESSIONS

Poster #12

EVALUATION OF SWIM-UP TECHNIQUE IN BACTERIAL LOAD REDUCTION AND SELECTION OF HIGHLY FUNCTIONAL SPERM

Heloisa Faquineti BSc, MSc student[1,2], Juliana Pariz PD fellow[1,2,3,4], Rosa Casemiro BSc[1], Bruna Zillig BSc student[1], Caroline Ranéa BSc, MSc student[1,2,3], Donald Evenson PhD5, Elaine Costa MD, PhD[1,2,3,4] and Jorge Hallak MD, PhD[1,2,3,4]

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(Presented By: Heloisa Faquineti, BSc, MSc student)

Poster #13

EFFECTS OF VITAMIN D SUPPLEMENTATION IN ASTHENOZOOSPERMIC SEMEN SAMPLES INCUBED IN ANAEROBIC CONDITIONS – AN INITIAL REPORT

Heloisa Faquineti BSc, MSc student[1,2], Juliana Pariz PD fellow[1,2,3,4], Bruna Zillig BSc student[1], Caroline Ranéa BSc, MSc student[1,2,3], Inari Ciccone BSc, MSc[1,2,3,4], Parviz Gharagozloo PhD5, John Aitken PhD[5,6] and Jorge Hallak MD, PhD[1,2,3,4]

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(Presented By: Heloisa Faquineti, BSc, MSc student)

Poster #14

USE OF DENSITY DISCONTINUOUS GRADIENT LABORATORY PROCEDURE TO REDUCE BACTERIAL LOAD AND TO SELECT HIGHLY FUNCTIONAL HUMAN SPERM

Bruna Zillig, Juliana Pariz PD fellow[1,2,3,4], Caroline Ranéa Bsc, Msc Student[5,6,7], Rosa Monteiro BSc[5,7], Heloisa Faquineti BSc, MSc student[5,7], Ivan Iori MD student[5,7], Donald Evenson PhD8, Elaine Costa MD, PhD[1,2,3,4] and Jorge Hallak MD, PhD[1,2,3,4]

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(Presented By: Bruna Zillig)

Poster #15

THE INDEPENDENT AND COMBINATORIAL EFFECTS OF CAG AND GGN REPEAT LENGTH POLYMORPHISMS ON HORMONAL, SEMINAL AND ANTHROPOMETRIC MEASUREMENTS IN YOUNG SOUTH AFRICAN MEN

Sean Patrick PhD[1], Elizabeth van Rensburg PhD[2], Natalie Aneck-Hahn DTech[3,4], Maria Bornman DSc[1], Paulina Farias PhD[5] and Christiaan de Jager PhD[1]

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(Presented By: Sean Patrick, PhD)

Poster #16

MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) REVERSIBLY PERTURBS BLOOD TESTIS BARRIER (BTB) IN PRE-PUBERTAL RATS

Richa Tiwary PhD, Jorine Voss PhD and John Richburg PhD

University of Texas at Austin

(Presented By: Richa Tiwary, PhD)

Poster #17

TSPO DRUG LIGAND FGIN-1-27 EFFECTS ON CHOLESTEROL ACCUMULATION, LIPID DROPLET FORMATION AND STEROID PRODUCTION IN STEROIDOGENIC CELLS

Jin-Yong Chung PhD[1], Haolin Chen PhD[2], Vassilios Papadopoulos DPharm, PhD, and DSc[3] and Barry Zirkin PhD[4]

[1]Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health; [2]Department of Anesthesiology, the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China and Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205; [3]Department of Pharmacology and Pharmaceutical Science, School of Pharmacy, University of Southern California, Los Angeles, CA 90089; [4]Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205

(Presented By: Jin-Yong Chung, PhD)

Poster #18

PHARMACOLOGICALLY INDUCED RECOVERY OF LEYDIG CELL TESTOSTERONE PRODUCTION IN HYPOGONADAL RATS: IMPLICATIONS FOR HYPOGONADISM AND SPERMATOGENESIS

Jin-Yong Chung PhD[1], Sean Brown MHS[2], Haolin Chen PhD[3], June Liu[2], Vassilios Papadopoulos DPharm, PhD, and DSc[4] and Barry Zirkin PhD[2]

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(Presented By: Jin-Yong Chung, PhD)

Poster #19

UNRAVELLING THE ROLE OF TRIM28 IN SPERMATOGENESIS

Joel Tan PhD and Daniel Messerschmidt PhD

IMCB, A*STAR

(Presented By: Joel Tan, PhD)

Poster #20

REDUCED SPERM QUALITY AND TESTICULAR DECLINE IN ACQUIRED NIACIN DEPENDENCY (ANDY) MICE WITH LOW TISSUE NAD LEVELS

Corey Swanson BS, Cedric Mannie, Miles Wandersee BS, Frances Angel, Kirsten Jensen BS, Ralph Meyer PhD and Mirella Meyer-Ficca PhD

Utah State University

(Presented By: Mirella Meyer-Ficca, PhD)

POSTER SESSIONS

Poster #21
MEHP-INDUCED INCREASE OF PERITUBULAR MACROPHAGES IN THE RAT TESTIS
Ross Gillette PhD, Richa Tiwary PhD, Jorine W Voss PhD and John H Richburg PhD
The University of Texas at Austin
(Presented By: Ross Gillette, PhD)

Poster #22
FUNCTIONAL CHARACTERIZATION OF ORGANOTYPIC PORCINE TESTICULAR ORGANOIDS IN MICROWELL CULTURE
Sadman Sakib BPharm[1], Aya Uchida MSc[1], Paula Valenzuela-Leon PhD[1], Yang Yu PhD[2], Hanna Valli-Pulaski PhD[3], Kyle Orwig PhD[3], Mark Ungrin PhD[1] and Ina Dobrinski Drmedvet, MVSc, PhD, Dipl ACT[1]
[1]Department of Comparative Biology and Experimental Medicine, University of Calgary Faculty of Veterinary Medicine, Calgary, AB, Canada; [2]Biomedical Engineering Graduate Program, University of Calgary, Calgary, AB, Canada; [3]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
(Presented By: Sadman Sakib, BPharm)

Poster #23
SPERMATOGONIAL METABOLISM AND AUTOPHAGY
Anna Laura Voigt DVM and Ina Dobrinski Drmedvet, MVSc, PhD, Dipl ACT
University of Calgary
(Presented By: Anna Laura Voigt, DVM)

Poster #24
THE POTENT IMPACT OF SPERM DNA FRAGMENTATION IN ASSISTED REPRODUCTION
Minghan Sun MD,PhD, Yi Wang PhD, Ke Dou MD, Xiaojian Zhang MD, Hailian Wang MD, Tiantian Lei MD and Kun Yu MD
Divsion of Reproductive Medicine, Department of Obstetrics and Gynecology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan, China
(Presented By: Minghan Sun, MD, PhD)

Poster #25
HIPK4 IS ESSENTIAL FOR MURINE SPERMIOGENESIS
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[1]RIMD, Osaka University; [2]Baylor College of Medicine
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[1]Brown University; [2]Pacific Northwest National Laboratory
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Nicholas Robert MSc[1], Eric Lague PhD[1] and Jacques J. Tremblay PhD[2]
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YanHe Lue MD, Darren Teramoto BS, Christina Wang MD and Ronald Swerdloff MD

Division of Endocrinology, Department of Medicine, Los Angeles Biomedical Research Institute and Harbor-UCLA Medical Center

(Presented By: YanHe Lue, MD)

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Nathalia de Lima e Martins Lara PhD[1], Carolina Felipe Alves de Oliveira[1], Rodrigo Ribeiro Resende[2], Richard Michael Sharpe[3], Samyra Maria dos Santos Nassif Lacerda[1] and Luiz Renato de França[1]

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Christine Zhou[1], Ninad Zaman[1], Yumbo Li[1], Vassilios Papadopoulos[2], Barry Zirkin[3] and Kassim Traore[1]

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(Presented By: Mahmoud Huleihel, PhD)

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Maram Abofoul-Azab PhD[1], Eitan Lunenfeld MD[2], Eliahu Levitas MD[3], Atif Zeadna MD[3], Johnny Younis MD[4,5], Shalom Bar-Ami PhD[4,5] and Mahmoud Huleihel PhD[6]

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Christiaan de Jager PhD[1], Sean Patrick PhD[1], Tanita Cronje MSc[2] and Natalie Aneck-Hahn DTech[3,4]

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Anita Sharma MBBS, MS (obstetrics & gynecology)[1], Mamta Meena MBBS, MS (obstetrics & gynecology)[1] and Prachi Sharma PhD (reproductive biology)[2]
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Gunapala Shetty PhD[1], Thien Phan BS[1], Guo Zhong PhD[2], Nina Isoherranen PhD[2] and Marvin Meistrich PhD[1]
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Erika Oliveira PhD[1], Gleide Avelar PhD[2], Rebeca Pompano PhD[3], Sangjo Shim PhD[3], Budhan Pukazhenthi PhD[1] and Nucharin Songsasen PhD[1]
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Nima Pourhabibi Zarandi MD[1], Guillermo Galdon MD[1], Olivia Cornett BS[1], Nicholas Deebel MD[2], Mark Pettenati PhD[3], Stuart Howards MD4, Stanley Kogan MD[2], Anthony Atala MD[2] and Hooman Sadri-Ardekani MD, PhD[2]
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Anukriti Singh Master of Science[1], Ellen K. Velte PHD[2], Christopher B. Geyer PHD[3] and Brian P. Hermann PHD[1]
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Darius Paduch MD, PhD[1], Stephanie Hilz PhD[2], Andrew Grimsom PhD[2], Peter Schlegel MD[3], Anne Jedlicka MS[4] and William Wright PhD[5]
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Vanessa Brouard PhD and Martine Culty PhD
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Roxanne Couture[1], Mustapha Najih BSc[1], Mohamed Touaibia PhD[2] and Luc J. Martin PhD[1]
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Kaori Nozawa PhD[1], Qian Zhang[2], Haruhiko Miyata[2], Zhifeng Yu[1], Darius Devlin[1], Ryan Matzuk[1], Masahito Ikawa[2] and Martin Matzuk[1]
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Chatchanan Doungkamchan MD[1], Yi Sheng MD, PhD[2], Kien Tran[1], Meena Sukhwani PhD[2] and Kyle E. Orwig PhD[2]
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[1]ECU; [2]UTSA
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Carolina Oliveira PhD student, Nathália Lara PhD, Luiz Renato França PhD and Gleide Avelar PhD
Federal University of Minas Gerais
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Rashin Ghaffari[1], Kristin Di Bona PhD[2] and John Richburg PhD[2]
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Parag Parekh PhD[1], Baraa Souman BS[2], Thomas Garcia PhD[3], Marvin Meistrich PhD[4], Gunapal Shetty PhD[5] and Marie-Claude Hofmann PhD[4]
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Darius Devlin BS[1], Kaori Nozawa DVM, PhD[2], Yoshitaka Fujihara PhD[2,3], Masahito Ikawa PhD[3] and Martin Matzuk MD, PhD[2]
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Pauline Roumaud PhD and Luc J. Martin PhD
Biology Department, Université de Moncton
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Klementina Fon Tacer PhD, DVM[1], Melissa J. Oatley[2], Tessa Lord[2], Jon M. Oatley[2], Jonathon Klein[1], Heather Tillman[3] and P. Ryan Potts[1]
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(Presented By: Klementina Fon Tacer, PhD, DVM)

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Amy Tran, MS, Martine Culty, PhD, and Renita Moradian
University of Southern California
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Sarah Munyoki BA[1], Adrienne Shami BS[2], Qianyi Ma PhD[2], Chris Green PhD[2], Jun Li PhD[2], Sue Hammoud PhD[2] and Kyle Orwig PhD[1]
[1]University of Pittsburgh; [2]University of Michigan
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Helio Chiarini-Garcia PhD[1], Reginaldo Martelo MD, PhD[2], Andre Lucas Caldeira-Brant MsC, PhD candidate[3], Fabíola Nihi PhD[3], Marcos LM Gomes PhD[4], Augusto B Reis MD, PhD[5], Edson T Samesima MD, PhD[5], Fernando M Reis MD, PhD[6] and Fernanda RCL Almeida DVM, PhD[3]

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(Presented By: Helio Chiarini-Garcia, PhD)

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(Presented By: Kien Tran, BS)

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DAZL IS A MASTER TRANSLATIONAL REGULATOR OF MURINE SPERMATOGENESIS

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(Presented By: Eugene Yujun Xu)

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THE MOUSE STRAIN MATTERS: SUCCESS OF IN VITRO SPERMATOGENESIS IS DEPENDENT ON GENETIC BACKGROUND OF DONOR MICE.

Joana M.D. Portela MSc[1], Callista L. Mulder PhD[1], Saskia K.M. van Daalen BSc[1], Cindy M. de Winter-Korver BSc[1], Sjoerd Repping Prof Dr [1], Jan-Bernd Stukenborg Assoc Prof [2] and Ans M.M. van Pelt Prof Dr [1]
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(Presented By: Callista Leonie Mulder, PhD)

When gonadotoxic treatment is required in prepuberty, boys often face subfertility in adult life. The spermatogonia present in prepubertal testis are sensitive to chemotherapy and radiation damage, leading to a medium to high risk of irreversible subfertility later in life depending on treatment type and dosage. To preserve fertility in these boys, a testicular biopsy can be obtained and cryopreserved to preserve unharmed spermatogonial stem cells (SSCs). Currently several techniques are explored that may allow for fertility restoration from cryopreserved testicular biopsies in the future. Production of sperm by autotransplantation of in vitro propagated SSCs or gas-liquid interphase testicular organ culture or are such experimental techniques. A testicular organ culture method resulting in generation of functional murine sperm was established by Sato and colleagues using transgenic mice with a C57BL/6J and C3H background (strain B6C3F2). However, it has previously been shown that the C57BL6/J is one of the least efficient mouse strains for in vitro proliferation of SSCs in cell culture, while strains with a DBA2 background are successfully used for this purpose. Therefore, we aimed to compare the efficiency of in vitro spermatogenesis by organ culture with testicular tissue originated from C57BL/6J donors and mice with a C57BL/6J and DBA2 background (B6D2F2) (4-8 days postpartum). To study the dependency of genetic background for in vitro sperm development success, testicular fragments from C57BL/6J (n=3) and B6D2F2 (n=4) were cultured in a gas-liquid interphase for the duration of 6 weeks. Germ cell differentiation and spermatogenesis progression were analyzed immunohistochemically. Although cultured testicular fragments from B6D2F2 demonstrated progression of spermatogonia to spermatocytes after 1 week, no further progression to more advanced states was observed. In contrast, round spermatids were developed in C57BL/6J cultured testis fragments from week 2, continuing to full spermatogenesis from week 4 onwards. In conclusion, in this study we found that testicular organ cultures originated from C57BL/6J mice support complete spermatogenesis in vitro, while spermatogenesis could not be effectively initiated in B6D2F2, even though the efficiency of SSC propagation in vitro from these mouse strains is known to be the reverse. Our results strongly suggests that the balance between proliferation and differentiation of SSCs in vitro is strain dependent.

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Harald Welter, Nils Dellweg, Carola Herrmann, Kim Dietrich, Bastian Popper, Henryk Urbanski, J. Ullrich Schwarzer, Frank-Michael Köhn and Artur Mayerhofer
(Presented By: Artur Mayerhofer, MD)

In the adult testis the testicular peritubular cell (TPC) is a predominant site of expression of the glucocorticoid receptor (GR; NR3C1). Yet the roles of GR-signaling in the regulation of TPCs, or possible roles in overall testicular functions, are unknown. We confirmed GR-expression in TPCs in adult human and rhesus monkey, as well as in adult mouse testes, by immunohistochemistry. While not found in TPCs of immature and prepubertal monkey testes, it became detectable around the onset of puberty. Adult TPCs are smooth muscle-like cells, which contract and relax and thereby transport sperm. Contractile abilities are linked to smooth muscle proteins, e.g. actin (ACTA2), and the extracellular matrix (ECM) protein elastin (ELN) may be also involved. As noted for GR, only around puberty became GR, ACTA2 and ELN detectable in monkey TPCs, or in the ECM of the tubular wall of monkey testes, respectively. To examine whether GR signaling may be linked to the regulation of ACTA2 and ELN, we studied isolated human TPCs (HTPCs) from adult men, which as we found, continue to express GR in vitro (RT-PCR; Western blotting). The GR agonist dexamethasone (DEX) induced GR-translocation from the cytoplasm into the nucleus within 1 h. It also caused elevation of the immunophilin FKBP5 (measured after 6 h; qPCR). Likewise, expression levels of ACTA and ELN were strikingly augmented (measured after 24 h; qPCR). The GR-antagonist RU486 blocked these actions. Other ECM markers, namely collagen I, or levels of the androgen receptor, were not affected, but IL6 and CCL2 were significantly lower (24h). Short term (6h) organo-typic incubations of mouse testicular fragments indicated that inhibitory actions of DEX on IL6 and CCL2 mRNA (qPCR) were fast and occur also in whole mouse testes. Taken together, our data indicate specific post-pubertal testicular roles of GR in TPCs. Glucocorticoid signaling in HTPCs involves maintenance of the contractile, smooth muscle-phenotype of these cells and a contribution to the immunological environment of the testis.
(Supported in part by grants from Deutsche Forschungsgemeinschaft (DFG) MA1080/23-2 and 27-1)

Poster #4
DIRECTING HUMAN INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATION TOWARDS LEYDIG AND ADRENAL CELLS

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(Presented By: Lu Li, PhD)

Reduced serum testosterone (T), or hypogonadism, affects millions of men. Hypogonadism has been found to be associated with conditions that include infertility, cardiovascular diseases, altered mood, fatigue, decreased lean body mass, reduced bone mineral density, increased visceral fat, metabolic syndrome, and decreased libido and sexual function. Reduced serum T is common in aging men where Leydig cells are becoming less responsive to LH referred to as primary hypogonadism, a condition that also occurs in 50% of men diagnosed with idiopathic infertility. Administering T-replacement therapy (TRT) reverses many of the symptoms of low T levels. However, TRT is linked

to side effects such as infertility, and increased risk of prostate cancer and cardiovascular disease. Thus, there is a need to obtain T-producing cells, which could be used to treat hypogonadism based on transplantation and reestablishment of T-producing cell lineage in the body. In humans, T is synthesized by Leydig cells (LCs) likely deriving from mesenchymal cells of mesonephric origin. Although mesenchymal cells have been successfully induced into LCs, the limited source and possible trauma to donors hinders their use in clinical therapies. Alternatively, human induced pluripotent stem cells (hiPSCs) that are highly expandable in cell culture and have the potential to differentiate into all somatic cell types become the emerging source of autologous cell therapies. We have successfully induced the differentiation of hiPSCs through mesoderm and mesenchymal cell progenitors into either human Leydig-like cells (hLLCs) or human adrenal-like cells (hALCs) under different culture conditions. Factors critical for the normal development of LCs were added to both culture systems. hLLCs (i) express all genes specific for Leydig cells and important for T biosynthesis, (ii) synthesize T, rather than cortisol (F), in response to dibutyryl-cAMP and 22R-hydroxycholesterol, and (iii) display ultrastructural features resembling LCs. In contrast, hALCs (i) express steroidogenic genes contributing to cortisol (F) biosynthesis and (ii) synthesize F rather than T in response to the same stimuli. These data suggest that under appropriate culture conditions hiPSCs can be driven to form either LCs or ACs. This bidirectional approach offers an insight into the differentiation of steroidogenic cells originating from the same fetal precursors and unveils new venues for cell therapy to treat hypogonadism.

Poster #5

MITOCHONDRIAL TSPO DEFICIENCY TRIGGERS RETROGRADE SIGNALING THROUGH DYSREGULATED CYTOSOLIC CALCIUM HOMEOSTASIS IN MA-10 MOUSE TUMOR LEYDIG CELLS

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(Presented By: Vassilios Papadopoulos, PhD)

The outer mitochondrial membrane translocator protein (TSPO) binds cholesterol with high affinity and is involved in mediating its availability for steroidogenesis. Recently, using the CRISPR/Cas9 system, we generated MA-10 mouse tumor Leydig cell sub-lines carrying a Tspo exon2-specific genome modification and wild-type Tspo (Fan et al. Endocrinology 2018; 159:1130). TSPO deficiency led to reduced dibutyryl-cAMP (dbcAMP)-stimulated steroid biosynthesis and increased esterified cholesterol-enriched neutral lipid accumulation, suggesting reduction in the import of the steroidogenic pool of cholesterol into mitochondria. Data obtained indicated that this is likely due to TSPO-mediated reduced mitochondrial membrane potential ($\Delta\Psi m$) via regulation of VDAC1/tubulin interaction. In addition, the reduced $\Delta\Psi m$ likely reflects a dysregulation and/or maintenance failure of basic mitochondrial function(s). To explore the consequences of TSPO depletion from mitochondria in the hormone-dependent steroidogenic MA-10 Leydig cells, we assessed the transcriptomic changes in TSPO-deficient compared to wild-type cells using RNA-seq. Generated gene expression profiles were validated using real-time PCR. We report herein that there are significant changes in nuclear gene expression in the Tspo mutant vs. control cells. The identified transcriptomic changes were mapped to several cellular signaling pathways, including calcium signaling. Intracellular calcium levels were measured using Fluo-8 Calcium Assay and confocal microscopy. Data obtained suggest that the loss of TSPO resulted in the dysregulation of cytosolic calcium homeostasis, a phenomenon controlled by mitochondria. This is a retrograde signaling pathway between the mitochondria and nucleus. In the Tspo mutant cells, intracellular calcium homeostasis is not properly regulated under either basal or ionomycin-stimulated conditions compared to WT cells. This phenomenon is more striking in the presence of dbcAMP that induces cholesterol transfer and steroid formation in WT cells. These observations are likely resulted from the dysregulation of some key members in the NFκB pathway, i.e, up-regulated Irf1, Nfkb1 and Stat1 and down-regulated Fos, Egr1 and Rel. In conclusion, TSPO regulates nuclear gene expression(s) through intracellular calcium homeostasis and signaling. This is the first evidence of compensatory response to the loss of TSPO with transcriptomic changes at the cellular level.

Poster #6

INTRAFLAGELLAR TRANSPORTER PROTEIN 27 (IFT27) IS ESSENTIAL FOR TRANSPORTING SIGNAL PEPTIDE PEPTIDASE-LIKE 2A (SPPL2A) TO PROCESS PRECURSOR A-KINASE ANCHOR PROTEIN 4 (PRO-AKAP4) TO MATURE AKAP4 FOR ASSEMBLY OF FUNCTIONAL SPERM

Qian Huang, Shiyang Zhang, Bernd Schröder and Zhibing Zhang
(Presented By: Qian Huang)

Sperm flagella are specialized motile cilia. In addition to the core "9+2" axoneme, accessory structures, including the fibrous sheath, are essential for normal sperm function. These flagellar proteins must be transported from cell bodies through a transport system, presumably intraflagellar transport (IFT), a conserved mechanism driven by kinesin-based anterograde transport and dynein-based retrograde transport. So far, twenty-two Ift genes, including Ift27, have been identified. It has been shown that IFT27 is not required for cilia assembly in somatic cells; however, male germ cell-specific Ift27 knockout mice were infertile with multiple morphological sperm abnormalities, including a disrupted fibrous sheath formation. Testicular level of 110 kDa pro-AKAP4, the precursor protein of mature 84 kDa AKAP4, an A-kinase anchor protein (AKAP) and a major component of the sperm fibrous sheath, was dramatically increased, and the mature AKAP4 was significantly reduced in conditional Ift27 mutant mice. Thus, IFT27 plays unique roles in sperm formation and function. A yeast two-hybrid screen using IFT27 as bait identified signal peptide peptidase-like 2A (SPPL2A) as a major binding partner. SPPL2A plays a role in protein cleavage through its catalase center. Like in the Ift27 conditional knockout mice, testicular level of pro-AKAP4 was significantly increased in the global Sppl2a knockout mice, but this change was not observed in the Sppl2a knock-in mice with a mutation of the catalase center, suggesting that SPPL2A is involved in pro-AKAP4 cleavage. In COS-1 cells transfected with a pre-AKAP4 expressing plasmid, a full-length pre-AKAP4 was detected alone with a 26 kDa cleaved peptide, presumably due to endogenous SPPL2A expression. The level of the 26 kDa cleaved peptide was significantly increased when the cells were co-expressed with exogenous wild-type SPPL2A, but not the mutant SPPL2A. Given that processing pro-AKAP4 occurs at the site of fibrous sheath assembly in the developing flagella, we propose that SPPL2A is transported by the IFT27 to the fibrous sheath assembly site to cleave pro-AKAP4 into AKAP4 for fibrous sheath assembly. Sppl2a mutant mice are fertile and some pro-AKAP4 is still processed in the Sppl2a knockout mice, we believe that pro-AKAP4 can also be cleaved by other proteases. Our studies revealed one mechanism for sperm fibrous sheath assembly to build functional sperm.

POSTER ABSTRACTS

Poster #7

TESTICULAR MACROPHAGES IN ANDROGEN INSENSITIVITY SYNDROME

Paula Aliberti Doctoral Student[1], Marco A Rivarola MD PhD[1], Alicia Belgorosky MD PhD[2] and Esperanza Berensztein PhD[1]
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(Presented By: Esperanza Beatriz Berensztein, PhD)

In rodents, testicular macrophages (tMΦ) play a role in supporting Leydig cell and spermatogonial stem cell functions, in addition to their immunosuppressive functions. Two subpopulations of tMΦ, interstitial and peritubular have been reported. A relationship between androgens and tMΦ in prostate and breast cancer has been proposed. We showed that patients with androgen insensitivity syndrome (AIS) start losing their germ cells in early childhood, while Leydig cell hyperplasia appeared after puberty (Aliberti P. et al, 2018). Our objective was to describe the distribution of macrophages throughout normal testis postnatal development and to compare it to the observed in testes of AIS patients. Twenty-three normal testicular tissues were grouped in 3 developmental stages: Infancy (n=9, median age 3.4 months, range 1.5mo-8mo), Childhood (n=7, 3.3 years, 1.3y-8y), and Puberty (n=7, 13.8 years, 9y-15.6y). Nine testicular tissues of AIS patients were divided in Childhood (n=5, 6.4 years, 1.8y-10.3y) and Puberty/Young-Adulthood (n=4, 19 years, 16.2y-23y). Expression of CD68 (MΦ marker) and CYP11A1 (steroidogenic-cell marker) by immunohistochemistry was performed. In normal testis samples, a significantly lower percentage of tMΦ was observed at Infancy (Mean±SD, 11.1%±4.3) than at Puberty (19%±4.8), without differences with Childhood (16.4%±7.7). The percentage of tMΦ did not differ between both AIS groups, Childhood (4.1%±2.3) and Puberty/Young-Adulthood (8.2%±5.5). A significantly lower percentage of tMΦ in AIS than their normal counterparts was found. Macrophage histology also differed within both conditions, with mostly rounded tMΦ were present in the normal samples while most were spindle shaped in the AIS samples. Leydig cell hyperplasia in Puberty/Young-Adulthood AIS testes was assessed by CYP11A1 positive cells. In conclusion, the population of tMΦ increases from birth to puberty in normal human testis. In addition to the histopathological changes described in testis of AIS patients, a decreased tMΦ subpopulation was found. Androgen action is necessary for the normal development and distribution of human tMΦ. A role of tMΦ in germ cell survival is proposed.

Poster #8

CRISPR/CAS9-MEDIATED STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR) KNOCKOUT IN MA-10 CELLS PROVIDES INSIGHT INTO RELATIONSHIP BETWEEN STAR AND TRANSLOCATOR PROTEIN TSPO

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(Presented By: Melanie Galano)

The steroidogenic acute regulatory protein (STAR) plays an essential role in inducing the transfer of cholesterol into the mitochondria for hormone-induced steroidogenesis. It has been shown that STAR acts at the outer mitochondrial membrane where it interacts with a large protein complex composed of cytosolic and mitochondrial proteins (transduceosome), which includes the outer membrane proteins translocator protein (TSPO) and voltage-dependent anion channel (VDAC). This interaction leads to increased transfer of cholesterol through the aqueous intermembrane mitochondrial space to the P450 side-chain cleavage enzyme (CYP11A1) at the inner mitochondrial membrane, the rate-limiting step of hormone-induced acute steroidogenesis. Although previous studies have shown that STAR and TSPO both play critical roles in facilitating cholesterol transport into the mitochondria, the exact relationship and interactions between these proteins have yet to be fully elucidated. Our previous studies showed that knocking out Tspo in MA-10 cells, which are hormone-responsive mouse tumor Leydig cells, affects hormone-induced formation and the synthesis and processing of STAR. Further studies using in cell co-immunoprecipitation through in situ proximity ligation assays revealed a direct interaction between STAR and TSPO after cyclic adenosine monophosphate stimulation. To allow us to further explore the relationship between STAR and TSPO, we generated STAR knockout (KO) MA-10 cells using the CRISPR/Cas9 system. We found that stimulation of STAR KO cells by the TSPO ligands, FGIN-1-27 and XBD173, which induce steroid formation, failed to induce progesterone production. These findings suggest that the absence of STAR disrupts TSPO-mediated steroid formation. Collectively, these results show that while STAR is dependent on TSPO for proper function, TSPO is also dependent on the presence of STAR for steroidogenesis. This work provides further evidence of the critical relationship between STAR and TSPO in steroid biosynthesis.

Poster #9

INFLUENCE OF ETHANOL CONSUMPTION ON THE CELL STRUCTURE OF SPERMATOGENIC EPITHELIUM AND SPERM

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(Presented By: Anastasiya Spaska, PhD)

Introduction: Chronic ethanol abuse stands out amongst etiopathogenic factors of men's infertility and erectile dysfunction. High sensitivity of germ cells to the influence of ethanol and its metabolites is well-known.
Objective: Determine cytological and ultrastructural changes in the testis and sperm in conditions of experimental chronic ethanol intoxication.
Methods: Study involved 30 mature laboratory rats. Group 1 received 30% ethanol (0.2 ml per 100g bodyweight daily) for 28 days. Group 2 served as controls. Histologic and electron microscopic examination of the testes and spermograms performed. Results: After chronic ethanol intake seminiferous tubules diameter decreased by 30%. A loss of germ cells in spermatogenic epithelium was observed. Number of primary spermatocytes decreased by 32%, secondary spermatocytes – by 38%, spermatids on 7th stage of development – by 21% versus control group. Volume of Leydig cells nuclei decreased by 22% assuming their degradation and lack of synthetic activity, cytoplasm was vacuolated, mitochondrial matrix enlightened, with reduced cristae. Ultrastructurally lamina propria of seminiferous tubules was twisted and thickened. Cytoplasm of Sertoli cells and spermatids showed loss of connection, proving disturbance of hemato-testicular barrier. Myoid cells had enlightened nucleoplasm, vacuolated cytoplasm, deformed organelles. Spermogram parameters showed 17% decrease in spermatozoa number per ml, compared to control group. The number of vital spermatozoa has dropped down to 68%, while number of pathological forms increased up to 38%. Defective head (no acrosome, a small head or a double head) and defective flagellum (curved or split) was quite common. The kinesisgrams were significantly worse than in control. Overall mobility and number of progressive sperm significantly decreased. On electron micrographs some spermatozoa indicated granular-fibrillar structures inside their nuclei marking immature chromatin condensation. Also missing acrosome, irregular shaped with enlarged subacromosomal space, lack of microtubules in flagellum and deformation of mitochondria was observed. Though alcohol intoxication affects genetic material, leads to impairment of penetration capacity of sperm as well as its motor activity.
Conclusion: Chronic ethanol abuse causes atrophic changes in testicles and infertility through impairment of spermatogenesis and sperm quality.
Keywords: testis, sperm, ethanol.

POSTER ABSTRACTS

Poster #10

DYNAMIC SUBCELLULAR MEMBRANE LIPID REMODELING IN HORMONE-INDUCED LEYDIG CELL STEROIDOGENESIS

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(Presented By: Sathvika Venugopal, PhD)

Cholesterol is the sole precursor for all steroids produced in mammals. During acute steroidogenesis, large quantities of cholesterol are trafficked from its intracellular stores to the cholesterol-poor organelle, mitochondria, to be converted to pregnenolone. Although steroidogenesis is extensively studied, the actual source organelle that stores and then mobilizes cholesterol for steroid production and the pathway that assists in trafficking this hydrophobic molecule to mitochondria are yet to be determined. Utilizing the domain 4 (D4) of the Perfringolysin O protein produced by Clostridium perfringens, which binds to high concentrations of cholesterol in membranes without cytotoxicity, we first determined the source organelle. Live cell imaging analysis in a Leydig tumor cell line and primary rat Leydig cells utilizing mCherry tagged D4 revealed release of a pool of cholesterol from the plasma membrane within 30 minutes of hormonal stimulation. These observations led us to conclude that the bulk of steroidogenic cholesterol destined for mitochondria originates from the plasma membrane during acute steroidogenesis. We further identified a pregnenolone-mediated feedback mechanism that stops excessive cholesterol movement from the plasma membrane and thus protects mitochondria from cholesterol-induced toxicity. A precise mechanism that facilitates rapid and sustainable movement of large quantities of cholesterol to the mitochondria had yet to be determined. Previous studies showing an increased interaction between the endoplasmic reticulum and mitochondria during acute steroidogenesis, led us to hypothesize that cholesterol from the plasma membrane enters the endoplasmic reticulum via a membrane association and thus reaches mitochondria by plasma membrane – endoplasmic reticulum – mitochondria associations called PAMs. These membrane associations and cellular signals are facilitated by a variety of lipid classes. Hence, we addressed this hypothesis by subcellular fractionation of hormonally induced and also hormonally induced but steroidogenesis-inhibited MA-10 cells subjected to lipidomics analysis utilizing mass spectrometry. The results obtained from this study support the notion that PAMs are the route for cholesterol movement from plasma membrane to the mitochondria. Further, we also noted a dynamic reorganization of multiple lipid classes that facilitate the membrane associations and cellular signals.

Poster #11

WHAT IS THE BETTER CULTURE MEDIA FOR IMPROVE THE MOTILITY OF ASTHENOZOOSPERMIC SAMPLES IN ANAEROBIC CONDITIONS?

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(Presented By: Caroline Ranéa BSc, MSc student)

Introduction: Limited protocols established in Assisted Reproductive Techniques employ different culture media to promote in vitro conditions of spermatozoa totally removed to seminal plasma. Currently, there is no time of incubation and culture medium formulated specifically with required nutrients to sperm maintenance, development and maturation, including motility capacity.

Objective: To compare two commercial culture media added in asthenozoospermic samples during different periods of in vitro incubation.

Methods: Were used 46 asthenozoospermic (PR<32%) seminal samples from voluntaries men (21-45 years-old). Seminal parameters were evaluated and divided in assays: Protocol I (n=28) - fresh samples classified as T0 (control group), T1 (1 hour incubation), T2 (2 hours incubation), T3 (3 hours incubation) and T4 (4 hours incubation). Protocol II included 18 seminal samples, processed by discontinuous density gradient or simple wash, added two different medium culture: "Continuous Single Culture Media" (CSCM) + 15% "Human Serum Albumin" (HSA) and "Human Tubal Fluid" (HTF) + 10% "Serum Substitute Supplement" (SSS). Samples were incubated at 37ºC/CO2 atmosphere (5%), seminal parameters, DNA integrity (SCSA®) and reactive oxygen species (ROS) were evaluated before and after incubation with medium culture. Were used ANOVA, Pearson's correlation and independent T-Student tests.

Results: In Protocol I, there were significant reduction in total motile number ($p \leq 0.001$), total progressive sperm number ($p \leq 0.001$), progressive motility ($p=0.003$), total motility ($p=0.05$) in T4 when compared with T0. There were negative correlation between incubation time, total motility sperm number ($r=-0.444; p \leq 0.001$), total progressive sperm number ($r=-0.328; p \leq 0.001$) and non-progressive sperm ($r=-0.181; p=0.36$). In Protocol II, was adopted 2 hours incubation (Table 1).

Conclusion: Incubation with CSCM+HSA culture medium demonstrated positive effects on sperm motility in asthenozoospermic seminal samples after 2 hours incubation. This culture medium can be applied in the Andrology routine, replacing HTF medium, and reducing procedures cost by up to 70%. Financial support: FAPESP (n° 2017/03599-1)/ Androscience.

Table 1. Semen parameters of asthenozoospermic samples incubed with HTF+SSS and CSCM+HAS.

	Fresh sample (T0)	2 hours (T2)	P value
HTF + SSS			
Immotility sperm (10^6 per ml)			
Mean;SD	71.55; 16.45	58.83; 21.22	0.052
Progressive motility (PR;%)			
Mean;SD	12.05; 11.44	19.83; 15.04	0.090
Total motility (PR + NP,%)			
Mean;SD	28.39; 16.38	37.11; 20.87	0.172
DNA Fragmentation (DFI; %)			
Mean; SD	47.21; 28.03	49.47; 23.30	0.815
ROS ($x10^4$ cpm/20^6)			
Mean; SD	22.80; 35.00	10.35; 13.84	0.425
CSCM+HSA			
Immotility sperm (10^6 per ml)			
Mean;SD	69.94; 18.09	58.83; 19.06	0.089
Progressive motility (PR;%)			
Mean;SD	8.33; 6.82	24.55; 13.97	<0.001
Total motility (PR + NP,%)			
Mean;SD	26.61; 18.10	41.17; 19.06	0.025
DNA Fragmentation (DFI; %)			
Mean; SD	5.47; 2.77	5.20; 2.43	0.781
ROS ($x10^4$ cpm/20^6)			
Mean; SD	22.75; 11,69	13.89; 11.39	0.176

Mean; Standard Deviation (SD); $p < 0.05$

POSTER ABSTRACTS

Poster #12

EVALUATION OF SWIM-UP TECHNIQUE IN BACTERIAL LOAD REDUCTION AND SELECTION OF HIGHLY FUNCTIONAL SPERM

Heloisa Faquineti BSc, MSc student[1,2], Juliana Pariz PD fellow[1,2,3,4], Rosa Casemiro BSc[1], Bruna Zillig BSc student[1], Caroline Ranéa BSc, MSc student[1,2,3], Donald Evenson PhD[5], Elaine Costa MD, PhD[1,2,3,4] and Jorge Hallak MD, PhD[1,2,3,4]

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(Presented By: Heloisa Faquineti, BSc, MSc Student)

Introduction: Laboratory techniques were frequently developed to optimize sperm quality to Assisted Reproduction procedures. Semen processing by Swim-Up (SW) method is a methodology applied in semen samples sent to Assisted Reproduction Techniques (ART), aimed to select highly motile sperm.

Objective: To evaluate the role of SW in bacterial load reduction and selection of highly functional sperm. Methods: Thirteen semen samples from voluntaries were included between January and July 2018. Samples were incubated with Human Tubal Fluid media® (HTF) in anaerobic atmospheric (5% CO2 at 36.7°C). After 1 hour, 1 ml of supernatant was centrifuged and the pellet resupendend with HTF. Were performed before and after SW: seminal analysis, evaluation of mitochondrial activity (3'3-diaminobenzidine stain), DNA integrity (SCSA® method), antisperm antibody test (Marscreen®) and microbiological analysis (Gonolab® for culture of anaerobic bacteria and R1® for culture of Mycoplasma spp). For statistical analysis was used T Student test and adopted $p<0.05$.

Results: After SW, there were a significant increase in progressive motility (61.6% ± 9.5 vs. 38.7% ± 14.9; $p<0.001$), total motility (73.9% ± 11.6 vs. 61.3% ± 13.4; $p=0.037$), reduction of antisperm antibodies (3.9% ± 4.3 vs. 10.8% ± 10.0; $p=0.046$) and sperm without mitochondrial activity (18.0% ± 11.6 vs. 38.6% ± 30.3; $p=0.049$). Six fresh samples have bacterial growth (50% Enterococcus spp. and 50% Staphylococcus aureus). The SW was effective in reducing 100% anaerobic bacteria and 90.90% Mycoplasma spp.

Conclusion: SW demonstrate to be effective laboratory methodology to select high fertility potential sperm, with progressive motility, reduction of antisperm antibodies and high capacity of energy production by mitochondrial activity. In addition, the reduction of bacterial load can be contribute to apply this technique in Assisted Reproduction procedures and, consequently, increase gestational rates. Key words: Microorganism; Semen; Seminal Quality; Seminal Processing; Sperm; Swim-Up. Financial support: Androscience, High Complexity Clinical and Research Andrology Laboratory

Poster #13

EFFECTS OF VITAMIN D SUPPLEMENTATION IN ASTHENOZOOSPERMIC SEMEN SAMPLES INCUBED IN ANAEROBIC CONDITIONS – AN INITIAL REPORT

Heloisa Faquineti BSc, MSc student[1,2], Juliana Pariz PD fellow[1,2,3,4], Bruna Zillig BSc student[1], Caroline Ranéa BSc, MSc student[1,2,3], Inari Ciccone BSc, MSc[1,2,3,4], Parviz Gharagozloo PhD[5], John Aitken PhD[5,6] and Jorge Hallak MD, PhD[1,2,3,4]

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(Presented By: Heloisa Faquineti, BSc, MSc Student)

Introduction: Vitamin D is a versatile signaling molecule with classic effects on bone, calcium and phosphate homeostasis that targets also male reproductive organs. In male reproductive tract, vitamin D is involved in reproduction functions and can be associated with increase of sperm motility.

Objective: To report vitamin D effects on low-motility sperm submitted to incubation in anaerobic conditions.

Methods: Six asthenozoospermic semen samples from volunteer subjects were included between September and November 2018. After semen routine initial analyses, samples were processed by simple centrifugation. Four samples were incubated with Continuous Single Culture Media® (CSCM) and calcitriol 10-6 U (1,25-dihidroxicholecalciferol; 1,25VD) and two samples were incubated with CSCM and cholecalciferol 10-12 M (actived 7-dehidrocolesterol; 7-DHC), at 37ºC in a CO2 atmosphere (5%) by two hours, and sperm motility was analyzed.

Results: Two samples (samples 2 and 3) incubated with 1,25VD demonstrated progressive (16.5% vs. 43.0%) and total (24.5% vs.54.0%) motility increase when compared to pre-incubation samples. In addition, progressive motility mean of samples incubated with 7-DHC was greater than pre-incubation progressive motility (30% vs. 33%) (Table 1).

Conclusion: In this pilot study, was reported role of vitamin D showing to be effective in sperm progressive and total motility improve, reinforcing previous studies that suggested its positive effects in spermatogenesis and semen quality of male subjects. This results will direct new studies by our group to unravel Vitamin D mechanisms in sperm physiology. **Keywords:** Incubation; Motility; Semen; Vitamin D. Financial support: Androscience, High Complexity Clinical and Research Andrology Laboratory

Table 1 – Means and percentage of semen samples progressive and total motility pre and post-incubation with 1,25VD and 7-DHC.

	PRE-INCUBATION		POST-INCUBATION	
	Progressive motility (%)	Total motility (%)	Progressive motility (%)	Total motility (%)
Sample 1 + 1,25VD	11.0	13.0	6.0	10.0
Sample 2 + 1,25VD	3.0	11.0	16.0	26.0
Sample 3 + 1,25VD	30.0	38.0	70.0	82.0
Sample 4 + 1,25VD	34.0	65.0	10.0	39.0
Mean	**19.5**	**31.75**	**25.5**	**37.0**
Sample 5 + 7-DHC	38.0	80.0	20.0	50.0
Sample 6 + 7-DHC	22.0	51.0	46.0	55.0
Mean	**30.0**	**65.5**	**33.0**	**52.5**

Poster #14

USE OF DENSITY DISCONTINUOUS GRADIENT LABORATORY PROCEDURE TO REDUCE BACTERIAL LOAD AND TO SELECT HIGHLY FUNCTIONAL HUMAN SPERM

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(Presented By: Bruna Zillig, BSc)

Introduction: Bacteriospermia is present in 15% male subfertility cases. For this, a laboratory procedure is required to remove bacteria present in semen sample. Laboratory semen processing, such as density discontinuous gradient, is a methodology applied in semen samples sent to assisted reproduction techniques (ART), with the objective of selecting

mobile sperm, removing debris, round cells and unfeasible sperm. However, sperm physiology characteristics and bacteria load reduction is no totally elucidated after these processes.

Objective: To evaluate the role of seminal processing by the Isolate® method in bacterial load reduction and selection of highly functional sperm in semen samples.

Methods: The present study has used 19 semen samples from volunteer patients. Samples were prepared using the Isolate® method. Fifty hundred milliliters of Lower layer, 0.5ml Upper layer and 1ml sample were added in a tube and centrifuged by 15 minutes at 1600 rcf. The pellet was resuspended in 0.5 ml culture medium. It was executed seminal analysis (sperm concentration, motility, sperm viability and morphology), functional tests [DNA Fragmentation Index (%DFI), High DNA Stainability (%HDS), mitochondrial activity, antisperm antibodies and reactive oxygen species dosage (ROS)] and microbiological analysis using Gonolab®, for anaerobic bacteria culture, and R1®, for Mycoplasma spp. culture. The Independent T test was used for the comparison of means and statistical analysis.

Results: Initial progressive motility was 25.31% and post-Isolate 8.45% (p=0.076). After seminal processing, a significant decrease in HDS (9.30% ± 5.07 vs 3.30% ± 1.70, p = 0.005) and increase in ROS (4.17%±4.27 vs. 20.16%±18.57, p=0.046) were observed. In 13 samples has growth bacteria, being 53.3% Staphylococcus aureus and 42% Enterococcus spp. Isolate was effective in bacterial load reduction, showing 88.88% efficiency by Gonolab and 0% in R1.

Conclusion: Seminal process was effective in reducing immature sperm and this could be applied in Assisted Reproduction techniques. In addition, the procedure was efficient in samples bacterial load reduction, except Mycoplasma spp., being a good laboratory tool to be used in the seminal samples of men with genitourinary tract infection, guaranteeing success in fertilization, since the reduction of bacterial load is fundamental to minimize effects such as embryonic malformation, congenital malformations or implantation failures. Financial support: Androscience

Poster #15

THE INDEPENDENT AND COMBINATORIAL EFFECTS OF CAG AND GGN REPEAT LENGTH POLYMORPHISMS ON HORMONAL, SEMINAL AND ANTHROPOMETRIC MEASUREMENTS IN YOUNG SOUTH AFRICAN MEN

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(Presented By: Sean Mark Patrick, PhD)

Introduction: The androgen receptor (AR) activates upon binding to testosterone and is involved in regulating androgen-related gene expression. The AR presents two polymorphic sites in exon 1, characterised by a different number of CAG and GGN triplet repeats. We tested the hypothesis that CAG and GGN lengths had independent and combinatory effects on hormonal, seminal and anthropometric measurements in young men from a malaria area, non-occupationally exposed to DDT.

Methods: An analysis was conducted on 528 Venda men (18-44 years; mean age=22) for CAG and GGN repeat characterization. Of these men, 291 were exposed to DDT through indoor residual spraying (IRS). Associations between anthropometric measurements, semen parameters and testosterone, with both the discrete and dichotomous number of CAG and GGN repeats, were evaluated by multiple linear and logistic regression, respectively. All models were run controlling for age, smoking, and DDT exposure. Transformation of dependent variables was applied when required to normalize their distribution.

Results and Discussion: Although CAG repeat length has been a significant positive predictor of height in other studies, this was not seen in our data. Weight showed significant and positive associations to long CAG and short GGN repeats analyzed separately, but not with the combined variable; i.e. men with long CAG repeats have a mean weight approximately 3 kg more than men with short CAG repeats (>25 vs. ≤25), and the mean weight of men with short GGN repeats is approximately 2 kg less than the mean weight of men with long GGN repeats (<13 vs ≥13). Mean total testosterone levels are almost two units lower in men with GGN<16, compared to men with GGN≥16. The fact that GGN repeats was significant as a weight predictor only when <13, while it was a significant predictor of testosterone levels only when <16, suggests that weight and hormones have different thresholds for an effect. Mean arm span was 4 cm longer in men with a long CAG repeat (>25). Regarding sperm motility, significant associations were seen with CAG repeats pointing to a decrease in progressive motility and a 2.5-fold increased risk of asthenozoospermia. Likewise, having both a long CAG and a shorter GGN (>20 and <16) showed an increased risk by 1.7 times of presenting with oligozoospermia. The hypothesis was confirmed and warrants further investigation into the combinatory impact of CAG and GGN repeats on body composition, and seminal parameters.

Poster #16

MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) REVERSIBLY PERTURBS BLOOD TESTIS BARRIER (BTB) IN PRE-PUBERTAL RATS

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(Presented By: Richa Tiwary, PhD)

The blood testis barrier (BTB) constituted by tight junctions between Sertoli cells creates an unique microenvironment for developing spermatocytes, is a well-studied target of numerous environmental toxicants. MEHP is the active metabolite of a widely used plasticizer (DEHP) in commercial products and has been recognized as a reproductive toxicant. Here, the influence of MEHP on BTB integrity is described as well as the signal transduction pathways that underlie this effect. Treatment of Post Natal Day (PND) 27 rats with 700 mg/kg MEHP for 24 hours perturbed the BTB integrity as indicated by a biotin tracer assay. Additionally, MEHP treatment induced transient surge of p44/42 Mitogen Activated Protein Kinase (MAPK)- JNK1/2, p38 and ERK protein levels; possibly instigated via the observed enhanced expression levels of pro-inflammatory cytokines-IL-6 and TNF□ as indicated by qPCR analysis. We further investigated that MEHP treatment of PND 27 rats with 700 mg/kg MEHP followed by a recovery period of 5 weeks could reverse the BTB disruption. Taken together, these findings indicate a role for the MAPK pathway in instigating the disruption of the BTB disruption after MEHP exposure; although this effect on the BTB was reversible.

POSTER ABSTRACTS

Poster #17

TSPO DRUG LIGAND FGIN-1-27 EFFECTS ON CHOLESTEROL ACCUMULATION, LIPID DROPLET FORMATION AND STEROID PRODUCTION IN STEROIDOGENIC CELLS

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(Presented By: Jinyong Chung, PhD)

Introduction and Objectives: Luteinizing hormone (LH) binding to the LH receptor initiates Leydig cell cAMP production and PKA activation, thus promoting the translocation of cholesterol to the inner mitochondrial membrane. Translocator protein (18 kDa TSPO), which binds cholesterol, is among the proteins involved in cholesterol translocation. Additionally, TSPO-specific ligands have been shown to induce changes in the distribution of intracellular cholesterol in astrocytes and fibroblasts. Our objective was to shed further light on mechanisms by which TSPO functions in Leydig cells, focusing on TSPO drug ligand stimulation of cholesterol distribution.
Methods: To determine whether FGIN-1-27 affects cholesterol accumulation and lipid droplet formation, MA-10 cells and Leydig cells isolated from Brown Norway rat testes were incubated with NBD-cholesterol in the presence of FGIN-1-27, and then with Nile-Red to stain lipid droplets. Adipocyte differentiation-related protein (ADFP), a protein that is highly expressed on the surface of lipid droplets, was analyzed by Western blotting. The effects of knockdown of adipocyte differentiation-related protein (ADFP), a protein that is a structural component of lipid droplet membrane, was assessed.
Results: When cultured with LH or the TSPO drug ligand, FGIN-1-27, steroid formation in both MA-10 cells and primary Leydig cells increased, associated with increased cholesterol accumulation and lipid droplet formation. Knockdown of ADFP resulted in the failure of MA-10 cells to produce steroid in response to LH or FGIN-1-27. Steroid production in response to FGIN-1-27 also was inhibited by the cholesteryl ester hydrolase inhibitor diethylumbelliferyl phosphate (DEUP), which affects the conversion of cholesteryl ester to free cholesterol in lipid droplets.
Conclusions: These results, taken together, suggest that FGIN-1-27, and thus TSPO, functions in steroid formation by stimulating cholesterol accumulation and lipid droplet differentiation, as well as in cholesterol translocation from lipid droplets into the mitochondria. This work was supported by: NIH grant R01 AG21092 (BZ), Natural Science Foundation of China grants 81471411 and 8074104 (HC), CIHR grants MOP125983 and PJT148659 (VP), and the John Stuffer Dean's Chair in Pharmaceutical Sciences (VP).

Poster #18

PHARMACOLOGICALLY INDUCED RECOVERY OF LEYDIG CELL TESTOSTERONE PRODUCTION IN HYPOGONADAL RATS: IMPLICATIONS FOR HYPOGONADISM AND SPERMATOGENESIS

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(Presented By: Jinyong Chung, PhD)

Introduction and Objectives: In Brown Norway rats, as in men, serum T declines with age as consequence primary hypogonadism. Currently, T replacement therapy (TRT) is used to treat primary hypogonadism, an approach that can have suppressive effects on spermatogenesis. This makes TRT inadvisable for men who wish to father children. Translocator protein (TSPO), a high affinity mitochondrial cholesterol-binding protein, is involved in cholesterol transfer from intracellular stores into mitochondria. We hypothesized that administering the TSPO-specific drug ligand FGIN-1-27 to hypogonadal rats would elevate serum T by stimulating the Leydig cells to produce higher levels of T, but in contrast to exogenous T administration, without reductions in LH or intratesticular T concentrations and thus without spermatogenesis suppression.
Methods: Rats of ages 3 and 21 mo. received FGIN-1- 27 via daily ip injection for 10 days. Serum and intratesticular fluid were collected for T measurement. To compare TSPO drug ligands to exogenous T administration, rats were administrated subdermal 2 cm T-containing silastic implants. After 10 days, serum and testicular fluid were collected for T measurement. To determine the T concentration required to maintain spermatogenesis, rats received subdermal T-containing implants of 1-24 cm length. After 8 weeks, intratesticular fluid was collected from one testis, and numbers of advanced spermatids were determined in the contralateral testis.
Results: Serum and intratesticular T levels were significantly lower in aged than young control rats. Both exogenous T and FGIN administration increased serum T level in old rats to that of young controls. With exogenous T, serum LH and Leydig cell T formation were suppressed, and intratesticular T was reduced to below the concentration required to maintain spermatogenesis. In striking contrast, the administration of FGIN-1-27 to old rats stimulated Leydig cell T formation but did not suppress LH, and resulted in increases both in serum and intratesticular T concentrations.
Conclusions: These results provide evidence that targeting TSPO with specific drug ligands might provide a means by which to increase serum T levels without administering LH-suppressive T and thus without a negative effect on spermatogenesis. This work was supported by grants from NIH (BZ), Natural Science Foundation of China (HC), CIHR (VP), and the John Stuffer Dean's Chair in Pharmaceutical Sciences (VP).

Poster #19

UNRAVELLING THE ROLE OF TRIM28 IN SPERMATOGENESIS

Joel Tan PhD and Daniel Messerschmidt PhD
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(Presented By: Joel Heng Loong Tan, BSc (Hons))

The role of epigenetics in spermatogenesis is becoming more apparent and male infertility is increasingly linked back to epigenetic defects. Tripartite motif-containing 28 (TRIM28) is an indispensible epigenetic transcriptional co-regulator that has been shown to regulate numerous biological processes including cellular differentiation. While little is understood about the role of TRIM28 in spermatogenesis, deleting it leads to testicular degeneration in mice, which warrants further investigation. In our laboratory, we observed that Trim28-heterozygous (Trim28Het) male mice became infertile prematurely, pointing to a likely haploinsufficiency phenotype of Trim28. Mating experiments confirmed this observation. As these mice grew older, their testes progressively shrank compared to the wild type. Histological analysis uncovered an increase in Sertoli cell-only tubules, suggesting that the size reduction could be attributed to the loss of germ cells. Since the degenerative

phenotype might be a consequence of systemic Trim28-heterozygosity, we generated and compared germ cell-specific and Sertoli cell-specific heterozygotes. We found that halving the amount of TRIM28 in the germ cells phenocopied Trim28Het testes, indicating a germ cell autonomous effect. Survey of the germ cell population in the seminiferous tubules revealed that heterozygous germ cells are progressively lost, beginning with the undifferentiated spermatogonia. Contrary to previous publication, which showed that TRIM28 was only expressed in mid-pachytene spermatocytes to early elongating spermatids, we detected Trim28 expression in undifferentiated spermatogonia. Based on these results, TRIM28 appears to have a function in these early cells of spermatogenesis where the stem cell population resides. Preliminary results suggest that TRIM28 regulates the fate of the spermatogonial stem cells.

Poster #20

REDUCED SPERM QUALITY AND TESTICULAR DECLINE IN ACQUIRED NIACIN DEPENDENCY (ANDY) MICE WITH LOW TISSUE NAD LEVELS

Corey Swanson BS, Cedric Mannie, Miles Wandersee BS, Frances Angel, Kirsten Jensen BS, Ralph Meyer PhD and Mirella Meyer-Ficca PhD
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(Presented By: Mirella Meyer-Ficca, PhD)

Humans are dependent on vitamins of the B3 group (niacin) as precursors for the synthesis of nicotinamide adenine dinucleotide (NAD). Despite wheat flour fortification with niacin, vitamin B3 deficiency remains a public health concern in certain population groups, such as the elderly, pregnant women and alcoholics that remain at risk of low NAD+ levels. Importantly, age-related NAD+ decline occurs despite food fortification and has been identified as a major contributor to aging associated health decline. NAD+ is a central cofactor for most metabolic redox-reactions, and furthermore is essential for the activity of various epigenetic regulator proteins (sirtuins and poly(ADP-ribose)polymerases proteins PARP1 and PARP2). PARP enzymes also facilitate efficient DNA repair. Using a genetic mouse modeI with acquired NAD deficiency (ANDY), we investigated physiological consequences of niacin/NAD+ deficiency for sperm quality. We tested the hypothesis that low NAD+ levels are associated with increased DNA damage in male germ cells and reduced DNA strand break repair capacity due to impaired PARP activity. Blood and testicular NAD+, NADH, NADP+ and NADPH levels were quantified in niacin deficient ANDY mice and normal controls. Reduced testicular NAD+ levels were associated with a reduction in poly(ADP-ribosyl)ation, indicating impaired PARP activity. Because reduced PARP activity is expected to result in diminished cellular DNA repair capacity, we quantified residual DNA strand breaks in sperm using comet assays. Prolonged NAD deficiency led to declining sperm production and sperm quality and ultimately resulted in testicular degeneration. Results of a study testing the hypothesis that the observed testicular decline is reversible upon restoration of normal NAD+ levels will be presented.

Poster #21

MEHP-INDUCED INCREASE OF PERITUBULAR MACROPHAGES IN THE RAT TESTIS

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(Presented By: Ross Gillette, PhD)

Background: Mono(2-ethylhexyl)phthalate (MEHP) is a well characterized Sertoli cell toxicant that is a metabolite of a ubiquitous plasticizer (DEHP). Peripubertal exposure to MEHP causes a decrease in the supportive capacity of Sertoli cells that leads to spermatocyte apoptosis, disruption of the blood-testis barrier, and infiltration of macrophages and neutrophils. Recently, a population of macrophages phenotypically distinct from those in the interstitium was described in mice. These testicular peritubular macrophages (ptMφ) aggregate on seminiferous tubules near the spermatogonial stem cell (SSC) niche and are speculated to stimulate their differentiation.
Objective: We hypothesized that if ptMφs do indeed stimulate SSC differentiation, an increase in their presence would occur to facilitate the recovery of spermatogenesis after MEHP exposure.
Methods: Male Fischer CDF344 rats were exposed to 700 mg/kg MEHP or corn oil (vehicle control) via oral gavage on PND 28 and euthanized by CO2 asphyxiation 48 hours, 1 week, 2 weeks, 8 weeks, or 10 weeks after exposure. The testes were removed, decapsulated, fixed in 4% paraformaldehyde, and subjected to whole tubule immunofluorescence for MHC-II, a ptMφ marker, and PLZF, an undifferentiated spermatogonia marker.
Results: ptMφs in rats were observed in the testis similar to those previously described in mice; MHC-II positive cells with a ramified morphology and extended processes which occasionally extended beneath the peritubular myoid cell layer and in close proximity to PLZF positive germ cells. Quantification revealed that, unlike in the mouse, their number did not increase through puberty. MEHP exposure increased their number three-fold at 48 hrs, which returned to baseline within 1 week. Interestingly, ptMφ primarily aggregated in sporadic dense clusters, which accounts for a large portion of the increase seen in the MEHP condition. Further, we demonstrate that an injection of clodronate liposomes prior to MEHP exposure prevents migration of macrophages into the testis, which could serve as a useful tool for future experiments.
Conclusions: We demonstrate that ptMφ are present in the PND 28 male rat testes and remain at a comparatively low level until PND 84. MEHP exposure induces a short-term increase in the localization of ptMφs to the seminiferous tubules. This outcome is consistent with the hypothesized reparative role of ptMφs in stimulating SSC differentiation, although more investigation is required.

Poster #22

FUNCTIONAL CHARACTERIZATION OF ORGANOTYPIC PORCINE TESTICULAR ORGANOIDS IN MICROWELL CULTURE

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(Presented By: Sadman Sakib)

Three-dimensional (3D) organoids resemble primary tissue and can serve as an in vitro platform to study cell-cell interactions, tissue development, and toxicology. However, development of testicular organoids (TO) with tissue architecture similar to testis has remained elusive. Here, we present a microwell centrifugal aggregation system to establish multicellular 3D TOs. Pre-pubertal porcine testicular cells were clustered and cultured in microwells for 5 days and then analyzed by immunofluorescence. The cells self-organized into spheroids (166.4±4.15μm, n=3, 10 TOs measured/replicate) with clearly delineated seminiferous epithelium (outside) and interstitium (inside) compartments. UCHL1+ve germ (GC) and GATA4+ve Sertoli cells (SC) were in the exterior compartment on a basement membrane (BM), with alpha-SMA+ve peritubular myoid cells (PMC) along the inside of the BM and CYP450+ve Leydig and CD31+ve endothelial cells at the core of the interior compartment. Similar TOs were also generated from mouse, macaque and human testicular cells. The BM contained fibronectin, indicating PMC function and SCs expressed claudin 11 and occludin. Germ cells in TOs had an attenuated response

to retinoic acid (1µM, 48hrs) with fewer Stra8+ve GCs in TOs (21.7±6.5, 50 GCs) compared to 2D culture (73.1±7.1, n=3, 100 GCs, p=0.0008); indicating TO tissue architecture modulates response to retinoic acid similar to testis in vivo. GCs in TOs had fewer autophagosomes (LC3B+ve puncta, a marker of cellular stress; 9.9±2.4puncta/cell) compared to GCs in 2D culture (20±6.1puncta/cell, n=3, 10 GCs, p=0.0001) indicating less cellular stress in GCs maintaining physiological cell interactions in TOs. TOs treated with 0.5, 1 and 1.5µM of di-(2-ethylhexyl) phthalate showed increased autophagy in a dose-dependent manner (DMSO:14.3±6.67puncta/cell<0.5µM:15.3±3.6puncta/cell<1µM:24.6±5.2puncta/cell<1.5µM:33.8±4.6 puncta/cell, n=3, 10 GCs, p<0.0001), demonstrating TO's potential for toxicity screening. Taken together, TOs recapitulate 3D organization of the mammalian testis and provide a platform for studying the function of GCs and somatic cells, and for screening drug toxicity in a cellular context representative of the testis in vivo. Supported by NIH/NICHD HD091068-01

Poster #23

SPERMATOGONIAL METABOLISM AND AUTOPHAGY

Anna Laura Voigt DVM and Ina Dobrinski Drmedvet, MVSc, PhD, Dipl ACT
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(Presented By: Anna Laura Voigt, DVM)

Spermatogonial stem cells (SSCs) reside within the population of undifferentiated spermatogonia. In higher mammals and humans, the isolation and expansion of SSCs in culture has so far met with limited success. Thus, a better understanding of the basic biology of these stem cells is crucial. Recent work has shown an increase in autophagy associated with elevated levels of reactive oxidative species (ROS) during culture of spermatogonia. As ROS are mainly produced during mitochondrial respiration, it is crucial to investigate the mitochondrial metabolism of spermatogonia. We hypothesize that removal of spermatogonia from the testicular microenvironment and maintenance in culture causes disturbance of mitochondrial metabolism resulting in the degradation of mitochondria, high ROS production, and mitophagy. As Sertoli cells and germ cells are in physiological tight interaction, we comparatively investigated Sertoli cells and spermatogonia harvested from 1-week old pig testes to define as first objective a mitochondrial and metabolic phenotype. Mitochondria were labeled with the outer mitochondrial membrane marker Tomm-20. 3D reconstruction of mitochondria via z-stack using confocal microscopy and analysis via electron microscopy revealed a unique morphology. Mitochondria appeared round, donut-shaped, and accumulated around the nucleus. Moreover, spermatogonia had a 19.3% ± 7.63 (n=4; p= 0.006) lower mitochondrial mass than Sertoli cells assessed via mean intensity of MitoTracker Green in flow cytometry. However, surprisingly the mitochondrial membrane potential in the population of undifferentiated spermatogonia appears to be significantly higher by 54.4 % ± 9.752 (n=3; p=0.03) than in Sertoli cells based on mean intensity of tetramethylrhodamine ethyl esther (TMRE) staining in flow cytometry. Results of oxygen consumption rate assessment obtained with the 24XF Seahorse analyzer, showed that undifferentiated spermatogonia have significantly higher basal respiration than Sertoli cells (24.31 amol/cell/s ± 3.25 vs 6.77amol/cell/sec ± 3.5 ; n=10; p<0.0001). Moreover, preliminary results of glycolytic flux assessment analyzed via mass spectrometry indicate a higher trend of lactate production over time in Sertoli cells. These findings indicate that prepubertal undifferentiated spermatogonia rely on oxidative phosphorylation, have highly active mitochondria, and potentially are not able to meet metabolic demands in vitro. Supported by NIH/NICHD HD091068-01.

Poster #24

THE POTENT IMPACT OF SPERM DNA FRAGMENTATION IN ASSISTED REPRODUCTION

Minghan Sun MD,PhD, yi wang PhD, ke dou MD, xiaojian zhang MD, hailian wang MD, tiantian lei MD and kun yu MD
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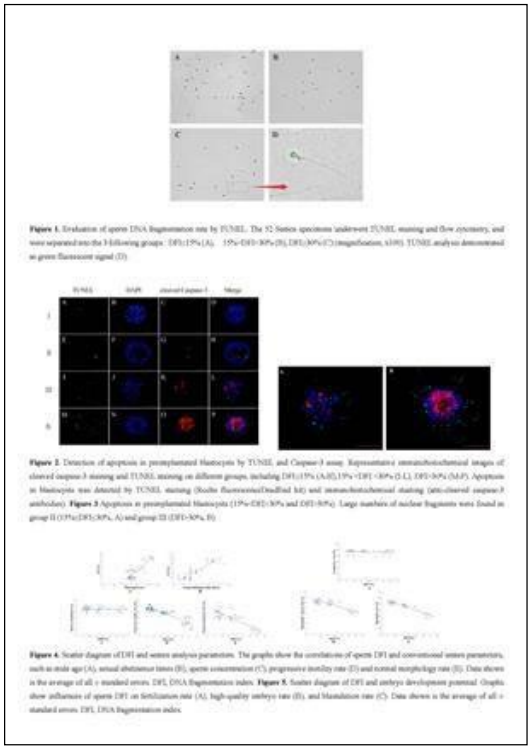
(Presented By: Minghan Sun, MD, PHD)

Objectives: In order to investigate the effect of DNA integrity on conventional semen parameters and pregnancy outcome in assisted reproduction, we systematically assessed sperm DNA fragmentation in intracytoplasmic sperm injections and embryo transfers (ICSI-ET).
Methods: A total of 52 couples underwent ICSI-ET treatment. According to the DNA Fragmentation Index (DFI), recipients were divided into three groups: DFI <15%, 15%≤ DFI≤30%, and DFI>30%. The correlation between sperm DNA integrity and potential embryo development was determined based on the three following factors: (1) conventional semen parameters, (2) analysis of clinical ICSI outcomes and (3) TUNEL and Caspase-3 assay.
Results: DFI was positively correlated with age and abstinence duration (r=0.67, p<0.05; r=0.73, p<0.05). However, it appeared negatively associated with forward motile sperm percentage (r=-0.86, p<0.05) and normal sperm morphological percentage (r=-0.69, p<0.05). Moreover, high levels of DFI were associated with both low blastulation rate and pregnancy rate (r=-0.87, r=-0.70, p<0.05). As for fertilization rate, no significant difference was observed (p>0.05). Further study on apoptosis indicated that, along with nuclear condensation, increased expression of TUNEL (+) cells and cleaved caspase-3 (+) cells were found in blastocysts with high DFI values prior to implantation.
Conclusion: Additionally to conventional semen parameters, sperm DNA damage could be a supplementary diagnostic index of male infertility. Because it activates apoptotic pathways, high level of sperm DNA fragmentation indicates a significant risk of embryonic death, leading to poor pregnancy outcomes. Key words Sperm DNA fragmentation, Sperm analysis, TUNEL, Caspase pathway, Assisted reproduction.



POSTER ABSTRACTS

Poster #25

HIPK4 IS ESSENTIAL FOR MURINE SPERMIOGENESIS

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(Presented By: J. Aaron Crapster, PhD)

Introduction: Spermiogenesis is a remarkable cellular transformation in which haploid round spermatids become elongated spermatozoa capable of motility and fertilization. Previous studies have revealed that many cytological changes are associated with this process, including formation of the actin-scaffolded acrosome/acroplaxome complex, nuclear reshaping, and flagellum assembly. However, the signaling pathways that coordinate these steps remain enigmatic. Here we demonstrate that an atypical member of the homeodomain-interacting protein kinase family, HIPK4, is essential for spermiogenesis and male fertility. Objectives. Our goal was to characterize the reproductive phenotypes associated with the loss of HIPK4.
Methods: Hipk4 expression was characterized by qPCR, in situ hybridization, and immunofluorescence studies. Hipk4 knockout mice were tested for their fertilization potential by mating, in vitro fertilization, and intracytoplasmic sperm injection (ICSI). Hipk4 mutant sperm were further evaluated for their ability to capacitate, undergo the acrosome reaction, and bind to oocytes. Electron microscopy and immunofluorescence studies were conducted on testis sections, isolated spermatids, and epididymal sperm. RNA profiling of Hipk4 knockout testes was performed by microarray analysis. HIPK4-dependent phosphorylation events in cultured fibroblast cells were identified by quantitative mass spectrometry.
Results obtained: HIPK4 is expressed in round and elongating spermatids. Male Hipk4 knockout mice are infertile, exhibiting oligoasthenoteratozoospermia, with no other overt physiological defects. HIPK4-deficient sperm exhibit diminished oocyte binding and are incompetent for in vitro fertilization, but produce viable pups via ICSI. Ultrastructural analyses reveal subtle acrosome/acroplaxome defects that arise in elongating Hipk4 mutant spermatids and lead to abnormal head morphology. Loss of Hipk4 does not dramatically alter testis-wide transcript expression; however, HIPK4 overexpression alters actin and Golgi dynamics in cultured somatic cells, and modulates the phosphorylation of known actin regulators.
Conclusions: Our findings establish HIPK4 as a critical regulator of spermatid head shaping and a potential contraceptive target.

Poster #26

CO-EXPRESSION OF SPERM MEMBRANE PROTEINS CMTM2A AND CMTM2B IS ESSENTIAL FOR ADAM3 LOCALIZATION AND MALE FERTILITY IN MICE

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(Presented By: Yoshitaka Fujihara, PhD)

Chemokines are signaling proteins that are secreted to induce chemotaxis during an immunological response. However, the functions of transmembrane-type chemokine-like factor (CKLF) and the CMTM (CKLF-like MARVEL transmembrane domain containing) protein family remain to be determined. In this study, we focused on the testis-specific mouse CMTM gene cluster (Cmtm1, Cmtm2a and Cmtm2b) and generated CRISPR/Cas9-mediated mutant mice to examine their physiological functions. Although Cmtm1 mutant mice were fertile, Cmtm2a and Cmtm2b double mutant mice had defects in male fertility due to impaired sperm function. We found that co-expression of sperm membrane proteins CMTM2A and CMTM2B is required for male fertility and affects the localization of the sperm membrane protein ADAM3 in regulating sperm fertilizing ability. This work was supported by Ministry of Education, Culture, Sports, Science and Technology (MEXT)/ Japan Society for the Promotion of Science (JSPS) KAKENHI grants (JP15H05573 and JP16KK0180 to Y.F., JP25112007 and JP17H01394 to M.I.); Japan Agency for Medical Research and Development (AMED) grant (JP18gm5010001 to M.I.); Takeda Science Foundation grants to Y.F. and M.I.; a Chubei Itoh Foundation grant to Y.F.; the Eunice Kennedy Shriver National Institute of Child Health and Human Development (R01HD088412 and P01HD087157); and the Bill & Melinda Gates Foundation (Grand Challenges Explorations grant OPP1160866).

Poster #27

SPERM RNAS AS NOVEL BIOMARKERS TO PREDICT MALE INFERTILITY AND TOXICANT-INDUCED TESTICULAR INJURY

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(Presented By: Enrica Bianchi, PhD)

Assessing male reproductive toxicity of environmental and therapeutic agents relies on testis and epididymis histopathology in a pre-clinical setting while in humans, assessment depends on semen and serum hormone analysis, both of which are poor indicators of sperm health and reproductive potential. Therefore, there is an urgent need to identify a novel, non-invasive and reliable approach to monitor environmental and therapeutic agents' effects on male reproductive health. Sperm RNAs are novel biomarkers to predict infertility and testicular toxicity; therefore, spermatozoal RNA content and mRNA sequence were analyzed in mouse, rat and human sperm samples to identify sperm transcriptomic similarities and differences across species. Semen specimens were collected from men aged 18 to 55 years with proven fertility, presenting for vasectomy to the Urology Division, and analyzed according to World Health Organization 2010 criteria. Rat and mouse semen specimens were collected from control animals via repeated needle punctures of the cauda epididymides. Sperm large and small RNAs were extracted after somatic cell lysis using an optimized sperm RNA isolation protocol. Sperm transcriptomic similarities and differences across species were identified using mRNA-sequencing. Bioinformatics analyses, including gene set enrichment analysis and Ingenuity Pathway Analysis, were used to investigate the biological function of all shared and differentially expressed transcripts across species. Mouse and rat sperm transcriptomes were more similar than either species compared to human. Transcriptome profiling identified 6684 similarly expressed transcripts within the three species that could be used to predict the clinical application of sperm biomarkers identified in toxicant-induced testicular injury animal models.

Poster #28

MRNA FRAGMENTS IN RAT SPERM ARE BIOMARKERS OF TESTICULAR INJURY DUE TO ETHYLENE GLYCOL MONOMETHYL ETHER EXPOSURE

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(Presented By: Angela R. Stermer, PhD)

Male reproductive toxicity poses a regulatory challenge due to the lack of simple robust analytical methods. RNA in sperm is reflective of the developmental process of the sperm, and changes in RNA composition could indicate toxic exposure. In order to identify highly predictive small RNA biomarkers of exposure in sperm, we used a combination of differential expression and LASSO regression after exposure to a known testicular toxicant, ethylene glycol monomethyl ether (EGME). Adult rats were exposed to 0, 50, 60 and 75 mg/kg EGME for 5 days then measuring small RNAs in sperm by next-generation sequencing 5 weeks

after exposure. Abundance of fragments from 11,000 transcripts were significantly changed with treatment. Then, a LASSO regression was performed on the significantly changed mRNA fragments. The LASSO regression was run in a bootstrap manner (100 iterations), recursively selecting groups of mRNA fragments that differentiated between EGME treatment and control with 90% accuracy. There were 547 mRNA fragments predicted 50 mg/kg EGME, 2744 mRNA fragments predicted 60mg/kg EGME and 7963 mRNA fragments predicted 75mg/kg EGME treatment. 91% (496) of the mRNA fragments that predicted lowest dose also predicted the highest dose, suggesting a common mechanism across the dose-curve. We looked at the selection rounds of these 496 mRNA fragments that were common predictors of all three treatments, to see if there were any that were repeatedly selected together. There were 9 mRNA fragments that were selected in either the first or second round in all of the 100 bootstrap iterations. There was consistency in the biological processes represented in enrichment analysis of all significantly changed mRNA fragments, the top 10 most significantly changed mRNA fragments, and LASSO identified mRNA fragments. The most represented biological processes were RNA processing, calcium signaling, GTPase signaling, proliferation and apoptosis. We conclude that the EGME mechanism may lead to highly reproducible changes in mRNA fragments. These mRNA fragments are highly predictive biomarkers of EGME in sperm.

Poster #29

INVESTIGATING THE ROLE OF LIVER RECEPTOR HOMOLOG 1 (LRH1, NR5A2) IN A CELL-SPECIFIC MANNER DURING MOUSE TESTIS DEVELOPMENT

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(Presented By: Kellie Suzanne Agrimson, PhD)

Introduction and Objectives: Liver receptor homolog 1 (LRH1) is an orphan nuclear receptor important for metabolic, developmental, and steroidogenic processes in both humans and mice. LRH1 is expressed in the mouse ovary, where it supports granulosa cell proliferation and follicle development. Granulosa and Sertoli cells play analogous supporting roles to oogenesis and spermatogenesis in the ovary and testis, respectively. However, the role of LRH1 has never been described in the mouse testis. **Methods:** Preliminary data suggest that Lrh1 is expressed in three of the main cell types in the testis: Leydig, Sertoli, and germ cells. Therefore, we have tested the role of Lrh1 in these cell types using CRE-Lox breeding schemes to ablate Lrh1 in specific cell lineages. Histological analyses were performed on testes from mice aged from embryonic day 15.5 to 1-year post-partum.
Results Obtained: Surprisingly, given the proposed role of Lrh1 in steroidogenesis, Leydig cell-specific knockout of Lrh1 in the mouse testis had no effect on spermatogenesis, with knockout mice fertile and able to produce pups when paired with a wildtype female. The Sertoli cell-specific knockout of Lrh1 resulted in fewer seminiferous tubules, significantly smaller testis size, and loss of germ cells, causing infertility. The germ cell-specific knockout testis progressively loses germ cells with age.
Conclusions: These observations imply that Lrh1 is required in both Sertoli and germ cells during mouse testis development for proper spermatogenesis. Based on the progressive loss of germ cells in the germ cell-specific knockout, we conclude that Lrh1 likely plays a role in SSC self-renewal. We are continuing to analyze phenotypic data and plan to perform LRH1 ChIP-seq and RNA deep sequencing of control and Lrh1 mutant testes to identify genes regulated by LRH1. Funding: This work is supported by NIH grants K12GM119955 and 5 R01 GM59152.

Poster #30

ISOLATION AND CHARACTERIZATION OF DIFFERENTIATING SPERMATOGONIAL SUBPOPULATIONS

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(Presented By: Rachel Lynn Gewiss)

Retinoic acid (RA) is a key mediator of several spermatogenic processes, including spermatogonial differentiation. This trigger for male germ cells to irreversibly transition from a relatively undifferentiated state into differentiating A1 spermatogonia normally occurs in patches along the seminiferous tubules. The pulsatile response to RA in successive parts of the seminiferous tubules results in asynchronous development of male germ cells, so that spermatozoa release occurs sequentially in space and time along the tubules and allows for continual male fertility. However, this heterogeneous nature of the testis make isolation and characterization of individual germ cell types difficult, particularly in the early stages of development immediately following spermatogonial differentiation. This issue is furthered by the fact that there are currently no molecular markers to distinguish the differentiating spermatogonial subpopulations from each other, and verification of these subpopulations must be performed with transmission electron microscopy. In order to mitigate this complexity within the testis, our lab developed a protocol to synchronize murine spermatogenesis using WIN 18,446 and RA. By depleting the testis of RA with WIN 18,446 and then causing synchronous differentiation via RA injection, germ cells proceed synchronously through development, and the times at which each of the differentiating spermatogonial subpopulations are present following RA injection have been previously characterized. By utilizing a PIWIL2-eGFP transgenic mouse line, we use fluorescence activated cell sorting in order to isolate germ cells away from Sertoli and Leydig cells, and isolate highly purified germ cell subpopulations. We have now used our synchrony and sorting procedures to compare the transcriptomes of undifferentiated spermatogonia and each of the differentiating spermatogonial subpopulations (A1 through B). Initial analysis with gene ontology has revealed that genes involved in meiotic processes are upregulated already in the Intermediate spermatogonia population, indicating that perhaps germ cells are already committed to undergoing meiosis by this developmental stage. Overall, this data provides an invaluable database for gene expression during each step of spermatogonial development that has previously been difficult to parse out from other cell populations within the testis. (Supported by HD10808 from NIH)

Poster #31

ACTIVIN A DEFICIENCY REVEALS TRANSCRIPTIONAL TARGETS AFFECTING FETAL MOUSE SERTOLI CELLS WITH IMPLICATIONS FOR STEROIDOGENESIS AND METABOLISM

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(Presented By: Katherine Loveland, PhD)

Normal murine gonad development involves dynamic production of TGFbeta superfamily signalling components, including elevation of transcripts encoding activin A in the fetal testis following sex determination. Previous studies showed activin A is required for normal Sertoli cell proliferation and testis cord morphogenesis, processes underpinning adult fertility. To elucidate the impact of activin A on somatic cells in the fetal testis, we first compared gene expression profiles in activin A-deficient mice (Inhba-/-) and their wildtype (WT)

littermates using a microarray (E15.5, pooled whole testis). Selected targets were further interrogated using a Fluidigm RT-qPCR-based analysis on E12.5, E13.5, E14.5 and E15.5 whole testes. RNA-seq was subsequently performed using FACs-purified somatic cells (from E13.5 and E15.5 WT, heterozygous and knockout littermates) isolated from Inhba-/- mice harboring an Oct4-GFP transgene. Further assessment of candidate target genes was conducted by exposing newborn whole testes to the pathway inhibitor, SB431542 in short-term cultures. Comparison with published cell type-specific datasets showed that most transcriptional changes arising from chronic activin A deficiency relate to genes expressed in Sertoli cells, however not all Sertoli cell genes are affected. Germ cells appear grossly unaffected, but both Sertoli and germ cells exhibit a remarkable accumulation of lipid droplets by E15.5, as previously reported in human testes with germ cell neoplasia in situ. Several activin A gene targets previously characterized in postnatal testes or in Sertoli cells of the mouse are shown to be similarly regulated in fetal testes, and several intriguing new activin A target genes are identified. We show for the first time a selective impact of activin A on steroidogenic genes expressed in fetal Sertoli cells, while those in Leydig cells are unaffected. These outcomes indicate how compounds that perturb fetal steroidogenesis, including endocrine disruptor chemicals, may intersect with activin and/or TGFbeta superfamily signaling to affect distinct cellular compartments and thereby impair testicular and germline development.

Poster #32

MECHANISMS OF TESTOSTERONE-INDUCED INSL3 TRANSCRIPTION IN MA-10 LEYDIG CELLS

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(Presented By: Nicholas Robert)

Insulin-like 3 (INSL3) is a small hormone produced by Leydig cells throughout embryonic and postnatal life. It is essential for the first step of testis descent during fetal development and for bone metabolism in adults. Despite these key roles, the molecular mechanisms regulating INSL3 expression remain poorly understood. So far, only a handful of transcription factors are known to activate the INSL3 promoter in Leydig cells including NUR77, COUP-TFII, SF1 and KLF6. In addition, INSL3 transcription is induced by testosterone (T) in an androgen receptor (AR)-dependent manner. However, the mechanisms of T/AR action remain unknown. The goal of this study was to shed new light on how T/AR acts on INSL3 transcription. Treatment of MA-10 Leydig cells with T resulted in a 2.7-fold increase in the activity of a -1137 to +11 bp human INSL3 luciferase reporter, as expected, and this activation was not affected by finasteride, a 5alpha-reductase inhibitor, indicating that T itself is involved. Next we used T coupled to BSA to determine if a transmembrane AR was implicated and found that only T and not T-BSA could strongly activate the INSL3 promoter confirming the involvement of an intracellular AR. This was further supported by the fact that pertussis toxin, an inhibitor of G protein-coupled receptor, had no effect on T-induced INSL3 promoter activity. Since T/AR can also act via intracellular signaling pathways, we tested the impact of a series of kinase inhibitors. Although we found that inhibition of ERK1/2 dramatically reduced basal INSL3 promoter activity, none of the inhibitors prevented the stimulatory effect of T. These data indicate that T acts via AR in a classical genomic pathway where T/AR translocates to the nucleus to activate gene expression. Since there is no AR binding site on the INSL3 promoter, we tested whether T mediates its effects by upregulating the expression of known activators of INSL3 transcription and/or by functionally cooperating with these activators on the INSL3 promoter. We found that T increases endogenous Sf1 and Nur77 mRNA levels by about 3 fold both in MA-10 cells and in primary Leydig cells. Furthermore, we observed that in the presence of T, the SF1- and NUR77-mediated INSL3 promoter activation was doubled. In conclusion, our results support a direct genomic action of T/AR in INSL3 transcription where T/AR cooperates with the nuclear receptors SF1 and NUR77 in addition to stimulating their expression. Supported by CIHR.

Poster #33

DEXRAZOXANE INDUCES REVERSIBLE SUPPRESSION OF SPERMATOGENESIS BY INDUCING APOPTOSIS OF DIFFERENTIATING SPERMATOGONIA AND PRIMARY SPERMATOCYTES

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(Presented By: YanHe Lue, MD)

Dexrazoxane (DRZ) is a drug approved to prevent anthracycline-induced heart toxicity in men. While effective in protecting the heart, DRZ injection alone has the potential to impair spermatogenesis in mice. To examine if DRZ could increase germ cell apoptosis and suppress spermatogenesis, we studied 20 adult male mice. Eight mice were used as control; 4 were given a single i.p. injection of DRZ (60mg/kg) and killed at 1 day after injection, and 8 received 4 doses of DRZ (60mg/kg) injections at 0, 1, 2 and 3 weeks, and killed at 1 day (n=4) and 110 days (n=4) after the last injection. Testis histology and sperm number in the cauda epididymides were examined. Germ cell apoptosis was detected by TUNEL assay and quantified as the number of apoptotic germ cells/100 Sertoli cells (AI). Compared to the control, the DRZ treatment at 1 day after a single dose significantly increased apoptosis (AI) of differentiating spermatogonia at stages I-III (Con:5.6±0.7 vs. DRZ:51.1±4.9), IV-VI (Con:1.7±0.3 vs. DRZ:58.6±5.9) and preleptotene spermatocytes at stages VII-VIII (Con:3.1±0.4 vs. DRZ:56.1±8.7) and dividing spermatocytes at stages XI-XII (Con:22.0±0.7 vs. DRZ:61.1±9.7). While no changes in body weight were seen (Con:26.8±1.4 vs. DRZ:26.5±1.4g), DRZ significantly decreased testis weight (TW, Con:96.4±4.1 vs. DRZ:46.0±4.8mg) and cauda epididymal sperm count (SC, Con:1.3±0.1 vs. DRZ:0.8±0.03 million/mg cauda). Histology showed that spermatocytes and round spermatids were markedly decreased, while elongated sperm remained in most of seminiferous tubules after 4 doses of weekly injections. The suppression of spermatogenesis caused by 4 repeated doses of DRZ was recovered at 110 days after the cessation of the injections (TW, 97.4±1.7mg; SC, 1.4±0.02 million/mg cauda). To examine the mechanisms of DRZ induced germ cell apoptosis, we performed immunoblot (SimpleWes®) using testicular lysates from the control and single dose of DRZ-treated mice, and demonstrated that the poly-ADP ribose polymerase (PARP-1) was significantly increased in the DRZ treated mice. We conclude that 1) DRZ induces reversible suppression of spermatogenesis by inducing apoptosis of differentiating spermatogonia and primary spermatocytes; 2) testicular PARP-1 elevation contributes to DRZ induced germ cell apoptosis. We recognize DRZ cumulative dosage is low and treatment duration is short. The longer experiments and studies to define mechanisms of suppression of spermatogenesis need to be investigated.

POSTER ABSTRACTS

Poster #34

NITRIC OXIDE IS AN IMPORTANT REGULATOR OF FETAL LEYDIG CELL TESTOSTERONE PRODUCTION

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(Presented By: Nathalia De Lima E Martins Lara, PhD)

Nitric oxide (NO) is produced by nitric oxide synthases (NOS) and is involved in several intra- and extra-cellular processes. In rodents, several studies have shown NO inhibition of testosterone (T) production in vitro and in vivo, while NOS inhibitors seem to up-regulate steroidogenesis. The inducible nitric oxide synthase (iNOS) enzyme is constitutively expressed in the adult testis, suggesting its regulatory role in testis function. Our previous studies showed that adult iNOS knockout mice (iNOS-/-) exhibit lower GC apoptosis, increased Sertoli (SC) and Leydig cell (LC) proliferation and final numbers, and, as a consequence, higher sperm production. In mice, adult LCs appear around Pnd10 (postnatal day). In the present study, we aimed to characterize T and NO levels in wild type (WT) and iNOS-/- mice, as well as the expression of important steroidogenic markers. The mice were evaluated during perinatal testis development, at e18.5 (embryonic day), Pnd1, Pnd5, Pnd10, Pnd15, Pnd20 and in adulthood, at Pnd70. Blood samples were collected after euthanasia, centrifuged and plasma stored at -80ºC for T and NO measurement, while testes were frozen for later qPCR or intratesticular T (ITT) and NO measurement. The qPCR data were analyzed by taking an average of four duplicates, normalized to β-Actin expression and corrected for the volume density of the cell type in which the marker gene was expressed. The significance level considered was $p<0.05$. Our results show that ITT levels were increased in iNOS-/- mice from e18.5 to Pnd10, concomitantly to a decrease in intratesticular levels of NO. Interestingly, a similar relation between plasmatic T and NO levels was observed during fetal life (e18.5), but it was mostly unchanged afterwards. It is worth mentioning that plasmatic T was lower in iNOS-/- mice at Pnd15, but no significant difference was observed in plasmatic NO at this age. Moreover, in most ages evaluated, the anogenital index, a marker of androgen exposure during fetal life, was significantly increased in iNOS-/- mice. However, our qPCR analyses showed that the mRNA levels of several steroidogenic-related genes are crucially reduced in iNOS-/- mice during the perinatal period (i.e. at e18.5, Pnd1 and Pnd5), such as StAR, 3β-HSD, Cyp11a1, 17β-HSD, 5α-reductase, aromatase, as well as the receptors for LHR, androgen and estrogen. Taken together, our data strongly suggest that fetal and adult LCs are differently affected by iNOS and NO.

Poster #35

REDOX-REGULATION OF HORMONE SENSITIVE LIPASE: POTENTIAL ROLE IN THE MECHANISM OF MEHP-INDUCED STIMULATION OF BASAL STEROID SYNTHESIS IN MA-10 LEYDIG CELLS

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(Presented By: Kassim Traore)

Introduction and Objectives: Mono-(2-ethylhexyl) phthalate (MEHP) is the active metabolite of di-(2-ethylhexyl) phthalate (DEHP), a plasticizer with endocrine disruptor activity that is widely used in the manufacturing industry. MEHP stimulates basal steroid biosynthesis in Leydig cells by molecular mechanisms that remain unclear. In the present study, we used MA-10 mouse tumor Leydig cells to study in vitro the effects of MEHP on ROS levels and the signal transduction pathways that mobilize free cholesterol to the mitochondria for steroidogenesis under basal conditions.
Methods: The effect of MEHP in basal progesterone production was assessed by treating MA-10 cells with 10, 50, 100 or 300 μM MEHP for 24 hrs. Progesterone production was quantified by enzyme-linked immunosorbent assay (ELISA). For qantification of phosphorylated HSL, MA-10 cells were incubated with 300 μM MEHP for 1, 3 or 6 hrs. ROS generation was assessed using DCFH-DA-derived fluorescence. In experiments in which the ROS scavenger N-acetyl cysteine (NAC) was used, MA-10 cells were treated with 300 μm MEHP or 5 mM NAC alone or in combination for 3 hrs. To measure oxygen consumption, cells were treated with MEHP (0-300 μM) for 24 or 48 hrs, and oxygen consumption was monitored using Clarke-type oxygen electrode. Mitochondria-derived superoxide was measured using the MitoSOX™ red mitochondrial superoxide indicator.
Results: MA-10 cells exposed to 0 - 300 μM MEHP stimulated basal progesterone production in a dose-dependent manner. The rise in progesterone levels correlated with an increase in the activating phosphorylation of hormone-sensitive lipase (HSL), the enzyme that frees cholesterol from internal storage, within 3 hours of exposure. StAR expression was also induced by MEHP. We explored the role of reactive oxygen species (ROS) signaling by co-treating MA-10 cells with the ROS scavenger N-acetyl cysteine (NAC). The results show that NAC blocked the activation of HSL and blunted MEHP-induced basal progesterone levels.
Conclusions: Together, these observations suggest that ROS generation by MEHP leads to activation of HSL and STAR that increase free-cholesterol bioavailability and progesterone biosynthesis.

Poster #36

INDUCTION OF MEIOTIC/POSTMEIOTIC STAGES AND SOMATIC CELLS ACTIVITIES BY PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) IN THREE-DIMENSION CULTURE FROM SPERMATOGONIAL CELLS OF IMMATURE MICE.

Mahmoud Huleihel PhD[1], Noy Bagdadi MSc[2] and Eitan Lunenfeld MD[3]
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(Presented By: Mahmoud Huleihel, PhD)

Introduction: Pigment epithelium derived factor (PEDF) is released as a soluble, monomeric glycoprotein, encoded by SERPINE-1 gene. Spermatogenesis occurs in the seminiferous tubules of the testis, which are avascular. It has been shown that cultures of human testicular peritubular cells constitutively secrete PEDF; and was suggested that PEDF may prevent vascularization of human seminiferous tubules. Recently, we showed the presence of PEDF in mouse spermatogenic cells and in Sertoli, Leydig and peritubular cells (unpublished data). In our previous studies, we showed the development of human and mouse sperm-like cells in three-dimension (3D) in vitro culture.
Objectives: To examine the possible involvement of PEDF in development of mouse spermatogenesis in vitro and to affect somatic cells activities.
Methods: Cells were enzymatically isolated from the seminiferous tubules of 7-day-old immature mice. Cells were cultured in metheylcellulose (as a three-dimension in vitro culture system), StemPro, LIF, GDNF, FGF, EGF and KSR, and in the presence or absence of PEDF (1-1000 ng/

ml). After 4-6 weeks of culture, the developed cells and colonies were collected and evaluated by immunofluorescence staining and qPCR analysis for cell markers of the different stages of spermatogenesis. Also, double staining and qPCR analyses were used to evaluate the production of growth factors from Sertoli, Leydig and peritubular cells.
Results: Our results showed development of colonies (proliferation) from isolated tubular cells in our system after 4 weeks of culture. Addition of PEDF to the isolated cells significantly decreased the percentage of the premeiotic cells (VASA), did not affect the meiotic cells (BOULE), but significantly increased the percentages of ACROSIN-positive cells compared to control (without PEDF) after 4 weeks of culture. On the other hand, addition of PEDF significantly increased the expression of LIF, GDNF and 3bHSD, but significantly decreased the expression of transferrin, PEDF and CSF-1, without significant effect on SCF and ABP compared to control as examined by qPCR analysis.
Conclusions: This is the first study to show a direct effect of PEDF on the induction of spermatogonial cells to meiotic/postmeiotic stages in vitro. It is possible to suggest that this effect could be directly on spermatogonial cells and/or through regulation of somatic cell functionality in vitro.

Poster #37
IDENTIFICATION OF PREMEIOTIC, MEIOTIC AND POSTMEIOTIC CELLS IN TESTICULAR BIOPSIES WITHOUT SPERM FROM SERTOLI CELL ONLY SYNDROME PATIENTS
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(Presented By: Mahmoud Huleihel, PhD)

Introduction: Sertoli cell only syndrome (SCOS) affects about 26.3%-57.8% of the azoospermic men. The seminiferous tubules of SCOS patients contain only Sertoli cells. Recently, it was reported that testicular biopsies from nonobstructive azoospermic (NOA) patients contain germ cells. Due to the fact that SCOS patients without sperm in their testicular biopsies have no therapy to father a biological child, in vitro maturation of spermatogonial stem cells (SSCs) isolated from testis of azoospermic patients is one of the new approaches for their possible future infertility treatment. Recently, the induction of human and mice SSCs proliferation and differentiation was demonstrated using different culture systems. Our group reported the induction of spermatogonial cells proliferation and differentiation to meiotic and post meiotic stages in mouse, rhesus monkey and prepubertal cancer patient boys, using 3D agar (SACS) and methycellulose (MCS) culture systems.
Objectives: The aim of the study was to identify the type of spermatogenic cells present in biopsies without sperm from SCOS patients, and to examine the possibility of induction spermatogenesis from isolated spermatogonial cells of these biopsies in vitro using 3D MCS.
Methods: We used 9 biopsies without sperm from SCOS patients. The presence of spermatogenic markers were evaluated by PCR and specific immunofluorescence staining analyses. Isolated testicular cells were cultured in MCS in the presence of StemPro enriched media with different growth factors. The development of colonies/cluster was examined microscopically. The presence of cells from the different stages of spermatogenesis before and after culture in MCS for 3-7 weeks were examined.
Results: Our results show that these biopsies showed the presence of premeiotic markers (2-7 markers/biopsy), meiotic markers (CREM was detected in 5/9 biopsies, LDH in 5/9 biopsies and BOULE in 3/9 biopsies) and postmeiotic markers (protamine was detected in 6/9 biopsies and acrosin in 3/9 biopsies). In addition, were able to induce development of meiotic and/or postmeiotic stages from spermatogonial cells isolated from 3 biopsies.
Conclusions: Our study shows for the first time the presence of meiotic and/or postmeiotic cells from biopsies without sperm of SCOS patients. Isolated cells from some of those biopsies could be induced to meiotic and/or postmeiotic stages under in vitro culture conditions.

Poster #38
THE IMPACT OF CHANGES IN MALARIA CONTROL STRATEGIES IN SOUTH AFRICA ON DDT EXPOSURE AND SEMINAL PARAMETERS
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(Presented By: Christiaan de Jager, PhD)

Introduction: Exposure to complex mixtures of endocrine disrupting chemicals (EDCs) are associated with adverse male reproductive health. In malaria-endemic areas, traditional huts are sprayed with 1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (DDT) while modern structures are sprayed with pyrethroid insecticides. DDT has estrogenic properties, and its metabolite DDE is a potent anti-androgen. With modernization of housing and DDT sourcing costs, spray programs have changed. This study investigated the effect of lower DDT levels and seminal parameters of men from DDT-exposed and non-exposed villages.
Methods: In a cross−sectional study conducted between 2012-2017, 431 young males, aged18-40 (24±4) years were recruited from six villages (three DDT-exposed – n=236; three non-DDT exposed - n=195) in a malaria endemic area in Limpopo Province, South Africa where DDT is used in indoor residual spraying. Exposure levels of DDT and metabolites were measured in blood plasma and a semen analysis conducted according to WHO standards. Linear regression models were examined to evaluate DDT/DDE effects on different reproductive outcomes. Seminal parameters were used as continuous variables in regression analysis and the dfbeta (dfß) statistic was determined.
Results and Discussion: Mean p,p'-DDT exposure levels in the 2012-2017 period were 0.92 ug/g (range 0.01 – 3.05) in the non-sprayed village and 0.92 ug/g (range 0.11 – 14.98) in the sprayed villages. In sprayed villages p,p'−DDE exposure levels were significantly lower from 216.9±210.6 ug/g (mean±SD) during 2003−2008 to 5.88±6.6 ug/g during 2012−2017 ($P < 0.001$). Men in the 2012-2017 group with p,p'−DDE levels between 0.26 and 2.25 µg/g are 2.6 times more likely to present with oligozoospermia than men with either lower or higher p,p'-DDE levels ($P<0.030$). No significant differences were seen in the sperm concentration and motility. Linear regression models indicated mean sperm head defects (= 0.01, $P = 0.05$) and tail defects were higher with increasing p,p'-DDT (= 0.25, $P < 0.01$) and p,p'-DDE (= 0.25, $P = 0.001$) exposure levels. Similar to findings in 2003-2008, current results point to weak associations between p,p'−DDE plasma concentrations

and sperm chromatin defects (%DFI) in the participants from sprayed villages (P < 0.010). In addition to DDT exposure, the role of complex environmental chemical mixtures should be investigated as health implications may include effects on the epigenome and metabolome.

Poster #39

NUMBER OF SPERMATOGONIA CELLS IN UNDESCENDED TESTES ARE LOWER THAN NORMAL REGARDLESS TO THE AGE AT ORCHIOPEXY; A CLINICAL VALIDATED PATHOLOGY REPORT

Stanley Kogan MD[1], Abinav Udaiyar BSc[2], Demetri Hodges BSc[2], Heather Barber BSc[2], Guillermo Galdon MD[2], Nima Pourhabibi Zarandi MD[2], James F Lovato MSc[3], Kimberly Stogner-Underwood MD[4], Shadi Qasem MD[4], Steve J Hodges MD[5], Anthony Atala MD[1] and Hooman Sadri-Ardekani MD, PhD[1]

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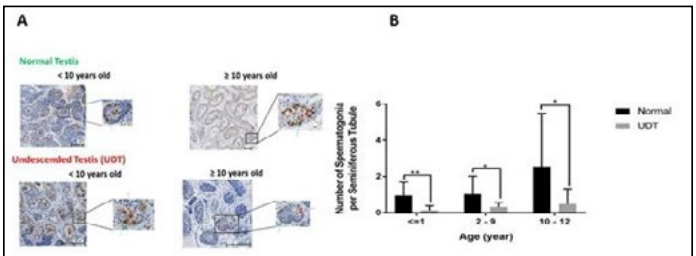
(Presented By: Stanley Jay Kogan, MD)

Introduction and Objectives: Failure of congenital testicular descent is the most frequent male genital abnormality, affecting approximately 1% of male births. Up to 20% of males with unilateral cryptorchidism experience fertility problems, increasing to 70% for patients with bilateral cryptorchidism. Orchiopexy is recommended within the first year of life. The main aim of this study was to quantify the number of spermatogonia at the time of orchiopexy in different age groups, as these are the most important cells to initiate and maintain spermatogenesis after puberty.

Methods: As part of our Institutional Review Board approved experimental testicular tissue banking to preserve fertility, testis biopsies were performed in patients who underwent bilateral orchiopexy because of undescended testes. Patients with retractile testes were excluded from this study. Testicular sections from paraffin blocks were prepared for PGP9.5 (UCHL1) antibody staining, an undifferentiated spermatogonia marker, in an automated clinical setting. The stained slides were scanned by NanoZoomer-XR Digital slide scanner. Using NDP.view2 software, the numbers of spermatogonia were counted. The results were compared with our previously established reference values from normal age matched pediatrics testes.

Results: The patients' ages ranged from 7 months to 12 years. Thirty-nine undescended testes were evaluated. The mean (95%CI) of total spermatogonia cells per seminiferous tubule was 0.14 (0.05 - 0.38), 0.32 (0.18 - 0.57) and 0.48 (0.17 - 1.32) in boys <1 (infantile), 1-10 (prepubertal) and 10-12 years old (peripubertal) respectively. In all age groups the number of spermatogonia cells were significantly less than normal value (p-values 0.005, 0.01 and 0.01 respectively) and the germ cell loss increased over time.

Conclusions: These data demonstrate the lower numbers of spermatogonia in bilaterally undescended testes regardless of the time of orchiopexy. The data indicate the possibility that storing a portion of the testis biopsy at the time of orchiopexy, propagating the number of spermatogonia stem cells (SSCs) in vitro and transplanting back the SSCs, potentially could increase the chance of fertility in these patients in the future.



(A) PGP9.5 staining (brown) of normal and undescended testes (UDT). Blue color (Hematoxyline) indicated nucleous. (B) Total number of spermatogonia in different age groups of normal and UDT subjects (p-value: * <0.05 and ** <0.01).

Poster #40

AGE RELATED PRESENCE OF SPERMATOGONIA IN KLINEFELTER SYNDROME PATIENTS: A CHANCE FOR BIOLOGICAL PATERNITY IN THE TESE NEGATIVE POPULATION

Nicholas Deebel MD[1], Guillermo Galdon MD[2], Kimberly Stogner-Underwood MD[3], James Lovato PhD[4], Stuart Howards MD[5], Stanley Kogan MD[1], Anthony Atala MD[1] and Hooman Sadri-Ardekani MD PhD[1]

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(Presented By: Nicholas Deebel, MD)

Introduction: Klinefelter syndrome (KS) has been defined as an X chromosome aneuploidy. The onset of puberty in KS patients is associated with the initiation of progressive testicular fibrosis, loss of spermatogonial stem cells (SSC), and infertility. However, focal areas of spermatogenesis have been observed in some patients. Given the recent success of KS SSC culture, it could be feasible to isolate, propagate, and differentiate these SSCs in vitro. The objective of this study was to identify which percentage of KS patients are still positive for spermatogonia on testicular biopsy. To address this question, a systematic review and meta−analysis of existing data is required.

Methods: A systematic Pubmed search was conducted. Exclusion criteria included: non−English language and review papers. Studies including data on the presence of spermatogonia in KS patients were included. Patients were subcategorized into the following age groups: Fetal/Infantile (age ≤1), Prepubertal (age 1<X≤10), Peripubertal/Adolescent (age 10<x<18) and Adult (age≥18). The presence of spermatozoa and spermatogonia on testicular biopsy along with the patients′ FSH, LH and testosterone were recorded.

Results: 722 papers were identified with double blind review returning 31 original articles with relevant information for meta−analysis on 376 patients. All the fetal/infantile and prepubertal groups were positive for spermatogonia while 42.7% and 48.5% of the peripubertal and adult groups respectively were positive for spermatogonia. Additionally, 26 of the 53 (49.1%) peripubertal and 37 of the 115 (32.2%) adult patients negative for spermatozoa were positive for spermatogonia. The mean FSH levels for combined peripubertal and adult patients were 22.0±2.60 for spermatogonia positive patients and 33.2±2.17 for spermatogonia negative patients (p= 0.001). Mean differences for LH and testosterone were statistically insignificant.

Conclusions: While azoospermia is a common finding in the KS patient population, many patients remain positive for spermatogonia. Given recent advances in KS SSC in vitro propagation, these cells could be used in future fertility interventions. This would offer many KS patients a chance at biological paternity.

POSTER ABSTRACTS

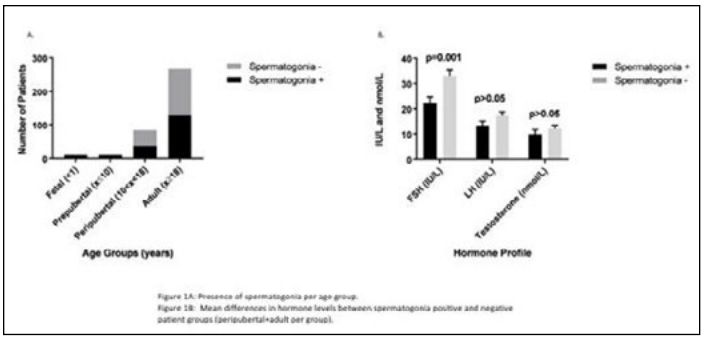

Figure 1A: Presence of spermatogonia per age group.
Figure 1B: Mean differences in hormone levels between spermatogonia positive and negative patient groups (peripubertal+adult oer group).

Poster #41
WITHDRAWN

Poster #42
RETINOIC ACID TREATMENT ENHANCES SPERMATOGONIAL DIFFERENTIATION IN IRRADIATED RATS ONLY WITH ANDROGEN SUPPRESSION
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(Presented By: Gunapala Shetty, PhD)

Introduction and Objectives: The extent of spontaneous recovery of spermatogenesis after radiation and chemotherapy is species dependent. In rats, there is a complete block in the spermatogonial stem cell to A1 transition and hormone suppression reversed this block. This suggested a failure in the somatic signaling for differentiation of type A spermatogonia, after cytotoxic insult. We reasoned that impairment of spermatogonial differentiation after irradiation may be due to retinoic acid (RA) deficiency in the tubules since the timing of the block in the spermatogonial differentiation coincided with the loss of spermatocytes and spermatids, which are the main intratubular sources of RA in the adult. Although RA may be produced in the interstitium or supplied via blood flow, peritubular myoid cells may act as a barrier to RA entry to the tubules. Furthermore, after spermatocytes are restored with GnRH-antagonist treatment, spermatogenesis continues to recover without any further treatment.
Methods: We used the irradiated Brown Norway rat model to investigate whether the impaired spermatogonial differentiation after irradiation is related to the deficiency of RA in the tubules. We measured the RA content in the testes of irradiated rats by mass spectrometry. We also tested whether exogenous RA enhanced spermatogenic recovery and the effect of additional androgen suppression treatment on the RA action.
Results: A dose of 4 Gy testicular irradiation, which eliminated nearly all of the germ cells 8 weeks later, in fact reduced the RA content per testis to about 16% of control. When rats were irradiated with 4.5 Gy, 2% of the tubules showed differentiation 8 weeks later. Hormone suppression with GnRH−antagonist treatment during weeks 5−8 after irradiation stimulated spermatogenic recovery in 57% of the tubules. RA treatment alone starting at 5 weeks after irradiation, did not enhance the recovery, as only 4% of the tubules showed differentiation. However, treatment with a combination of GnRH−antagonist and RA further enhanced the percentage of tubules with differentiated germ cells to 84%.
Conclusions: The failure of RA alone to stimulate spermatogonial differentiation and the enhanced differentiation when RA was combined with androgen suppression, supports the hypothesis that androgen suppression enables access of exogenous RA to the tubular compartment, allowing spermatogonial differentiation, at the time when the intratubular source of RA is depleted.

Poster #43
SHORT-TERM CULTURE OF CAT SEMINIFEROUS TUBULES IN A MICROFLUIDIC DEVICE
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(Presented By: Erika Oliveira, DVM, PhD)

Testicular tissue freezing has been proposed for fertility preservation in pre-pubertal boys, and cryopreserved tissue must undergo a maturation process to restore sperm production. One approach to obtain haploid cell from thawed testicular tissue is via in vitro maturation, and different culture systems are, therefore, being investigated. Recently, full mouse spermatogenesis in vitro was reproduced in an organotypic culture system, but the process was limited to the peripheral areas of the tubules. It is known the absence of circulating oxygenated blood supply in a static organ culture system gives rise to focal areas of hypoxia and/or necrosis. To overcome this, microfluidic (MF) technologies were incorporated into culture systems to provide conditions comparable to the vascular flow in vivo. We proposed to investigate a testis-on-a-chip system using the domestic cat as a model. Fragments of testis tissue from four domestic cats (5mo to 2y) were mechanically dissociated and groups of seminiferous tubules (8 groups of SeTs/cat) were cultured for 1 wk (34˚C, 5% CO2) on top of agarose block (AB) (Control) or in a MF device in Dulbecco's Minimum Essential Medium (DMEM), supplemented with 10% knockout serum replacement (KSR), FSH 10 ng/ml, LH 10 ng/ml, and retinoic acid (RA) 1 µM. For the MF system, culture medium was loaded into a syringe attached to a Newton syringe pump to maintain constant flow rate through the tissue: 0.2 µl/min (slow flow rate, SFR) or 4 µl/min (fast flow rate, FFR). Histological analysis of fresh SeTs revealed a preserved population of germ cells in different stages of differentiation up to pachytene primary spermatocytes. After 1wk SeTs kept under SFR and FFR conditions presented typical testicular arrangement, especially the SeTs located in the periphery, similarly to the SeTs cultured in AB. A more detailed histological analysis of the treatments revealed tubules with morphologically normal cells up to pachytene. There were few dispersed areas with cell vacuolization and shrunk nuclei in all systems. However, contrary to AB, MF (both SFR and FFR) was able to sustain cell division based on the presence of mitotic figures. The present use of the device, therefore, would provide a valuable foundation for future improvement of culture conditions for testis tissue and organ culture methods.

Poster #44
3−DIMENSIONAL HUMAN TESTICULAR ORGANOID SYSTEM FROM KLINEFELTER (47XXY) TESTICULAR CELLS SUPPORTS IN VITRO HAPLOID GERM CELL FORMATION
Nima Pourhabibi Zarandi MD[1], Guillermo Galdon MD[1], Olivia Cornett BS[1], Nicholas Deebel MD[2], Mark Pettenati PhD[3], Stuart Howards MD[4], Stanley Kogan MD[2], Anthony Atala MD[2] and Hooman Sadri-Ardekani MD, PhD[2]
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(Presented By: Nima Pourhabibi Zarandi, MD)

Introduction: Klinefelter Syndrome (KS) is typically defined as 47, XXY in a male patient. The onset of puberty in KS patients is associated with progressive testicular fibrosis, loss of spermatogonial stem cells (SSC), and impaired fertility. Previous work has demonstrated the ability to propagate in vitro SSCs in 2D cell culture. The objective of this study was to use propagated KS SSCs to form a functional 3−Dimensional Human Testicular Organoid system (3D HTO) as a means of establishing a novel infertility treatment for KS patients.

Methods: KS testicular cells from our previous 2D culture system were thawed and recovered for a week. 3D HTOs were formed by placing 10,000 cells per well in ultra−low attachment round bottom plates for 48 hours. After the formation, differentiating media was used for three weeks. Viability and structure of the HTOs was assessed with bright field and confocal microscopy as well as live/dead staining and ATP assays. Both qPCR and dPCR were used to confirm the presence of spermatogonia, Sertoli, Leydig and peritubular cells during all stages. Finally, stimulated and unstimulated testosterone production from the HTOs was measured. The presence of any haploid cell was assessed by post meiotic germ cells marker (PRM1) and FISH for chromosomes X/Y/18. The organoids were assessed at day 2, 9, 16, and 23.

Results: Well−defined spherical HTOs were formed after 48 hours. Live/dead staining remained stable while ATP assays showed an initial decline due to transition from 2D to 3D system. PCR confirmed the presence of the four major testicular cells types. HTOs produced testosterone constantly. Furthermore, SSC differentiation was shown in each stage of the experiment by gene expression (ZBTB16 for undifferentiated, DAZL for differentiating, SYCP3 for meiotic and PRM1 for post meiotic germ cells). After 3 weeks, at least 12% of cells in HTOs were haploid (X/18 or Y/18).

Conclusions: This is the first study to demonstrate the ability to form stable and viable HTOs from human KS testicular cells. Furthermore, this system was able to functionally maintain four major testicular cell types and differentiate SSCs to post−meiotic cells. Future studies will focus on collecting and differentiating viable haploid germ cells for use in ICSI therapy.

Poster #45

SINGLE-CELL TRANSCRIPTOMES OF PROSPERMATOGONIA REVEAL HETEROGENEITY IN THE RA RESPONSE IN THE NEONATAL MOUSE TESTIS

Anukriti Singh Master of Science[1], Ellen K. Velte PHD[2], Christopher B. Geyer PHD[3] and Brian P. Hermann PHD[1]

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(Presented By: Anukriti Singh, B.Tech)

Mammalian spermatogenesis begins with the segregation of prospermatogonia into type A undifferentiating and differentiating spermatogonia by postnatal days 3-4 (P3-4), which temporally coincides with the onset of retinoic acid (RA) signaling. Spermatogonial stem cells (SSCs) are a subset of undifferentiated spermatogonia that sustain spermatogenesis through the balanced production of progeny that will either retain stemness (self-renew) or initiate the process of differentiation by acquiring the ability to respond to RA. The mechanisms underlying differential spermatogonial competency for RA response are unknown. Using both in vitro and in vivo pharmacological approaches, we previously determined that CYP26-mediated RA catabolism prevents the majority of neonatal prospermatogonia from responding to RA. However, a subset of prospermatogonia representing putative SSC precursors are RA-insensitive and protected via a CYP26-independent mechanism. To explore differential RA responsiveness, we performed single-cell RNA-Seq on 34,153 testis cells from P1.5 mice treated with RA or DMSO. This analysis confirmed that only a subpopulation of prospermatogonia responded to RA by inducing known RA-regulated genes (e.g., Cpm, Cypb26a1, Cyp26b1, Rarb, Stra8). RA-responsive prospermatogonia (n=247) (Stra8+ or Sod3+ or Rarb+) exhibited elevated expression levels of 27 genes involved in G2/M DNA Damage checkpoint regulation (Cks1b, Top2a, Ccnb1), GADD45 Signaling (Ccnd2, Ccnb1) and Wnt/β-catenin signaling (Sox4, Rarb, Ubc). Only 3 genes were elevated in prospermatogonia (n=125) that did not exhibit an RA response and they were involved in EIF2 signaling (Rpl6). Collectively, these findings reveal multiple mechanisms, such as CYP26B1-mediated catabolism and absence of RA receptor expression, that protects subpopulations of prospermatogonia, including putative SSCs, from RA-induced differentiation.

Poster #46

INTERACTION BETWEEN MONO-(2-ETHYLHEXYL) PHTHALATE AND ALL-TRANS RETINOIC ACID ALTERS DEVELOPMENT OF EX VIVO CULTURED FETAL MOUSE TESTIS

Daniel Spade PhD and Susan Hall

Brown University, Department of Pathology and Laboratory Medicine

(Presented By: Daniel Spade, PhD)

Exposure to phthalate esters (phthalates) produces fetal testicular toxicity consisting of reduced testosterone production, altered seminiferous cord development, induction of multinucleated germ cells, and germ cell death. The anti-androgenic effects of phthalates vary across species more than multinucleated germ cell induction or changes in seminiferous cord diameter, suggesting that mechanisms in addition to disruption of testosterone production must contribute to phthalate toxicity. We have previously shown that exogenous all-trans retinoic acid (ATRA) reduces seminiferous cord number, alters expression of genes related to sex determination, and induces expression of the ovarian protein FOXL2 in cultured rat fetal testes, and that these effects are modulated by simultaneous administration of mono-(2-ethylhexyl) phthalate (MEHP). We hypothesized that disruption of retinoic acid signaling is a mechanism of phthalate toxicity in mice, as well as rats. To test this hypothesis, mouse fetal testes were isolated on gestation day 14, cultured ex vivo with 10^(-6) to 10^(-4) M MEHP, 10^(-6) M ATRA alone, or 10^(-6) M ATRA plus 10^(-6) to 10^(-4) M MEHP. Seminiferous cord development was assessed by quantifying cord number per section in histological sections of cultured testes. FOXL2 expression was assessed by immunohistochemistry. As previously observed in the rat, co-exposure to ATRA and MEHP resulted in a non-linear dose-response in reduction of seminiferous cord number, indicating an interactive effect of ATRA and MEHP on cord development. Further, elevated FOXL2 staining was observed in testes from both ATRA-only exposure and ATRA-MEHP co-exposure. These results provide evidence that MEHP and ATRA interact to disrupt mouse fetal testis development and support the hypothesis that retinoic acid signaling is a target for phthalate toxicity in mice.

Poster #47

THE ROLE OF RNA BINDING PROTEIN ADAD2 IN MALE MEIOSIS

Lauren Chukrallah, Gabriella Acoury and Elizabeth Snyder PhD

Rutgers the State University of New Jersey

(Presented By: Lauren Chukrallah)

Meiosis, the specialized cell division responsible for genetic recombination, is an essential, germ-cell-specific process. Successful meiosis is necessary for fertility and requires a unique set of molecular mechanisms including special RNA biogenesis events. Many of these events are mediated by meiosis-specific RNA binding proteins (RBPs). One such RBP, adenosine deaminase domain containing protein 2 (ADAD2), is expressed predominantly in meiotic spermatocytes and displays a developmentally regulated pattern of expression. Immunofluorescence in adult mouse testis showed ADAD2 is diffusely cytoplasmic in early pachytene and then forms perinuclear foci by late pachytene. To interrogate the function of ADAD2, a global Adad2 knockout mouse allele was produced via CRISPR-Cas9 mutagenesis of the endogenous Adad2 locus. This created a single nucleotide deletion which resulted in a frameshift, abrogating protein production. Adad2 knockout males are infertile with significant loss of post-meiotic germ cells. Given its meiotic expression and early post-meiotic germ cell defects in the mutant, the impact of ADAD2 loss on meiosis was assessed. Histological evaluation of Adad2 knockout testes revealed a trend toward decreased MII spermatocytes. To more accurately assess meiotic progression, immunocytochemical analyses of meiotic cell spreads from ADAD2 knockout mice were performed. These analyses demonstrated increased frequency of early pachytene spermatocytes and decreased

frequencies of late pachytene and diplotene spermatocytes. These data indicate that ADAD2 is required for optimal meiotic progression. A this defect coincides with a key pachytene progression checkpoint future work will focus on quantifying markers of the pachytene checkpoint failure to investigate whether the checkpoint is being triggered in Adad2 knockout spermatocytes.

Poster #48

ABERRANT GENE EXPRESSION BY SERTOLI CELLS IN INFERTILE MEN WITH SERTOLI CELL-ONLY SYNDROME

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(Presented By: William Wright, PhD)

Sertoli cell-only (SCO) syndrome is a severe form of human male infertility seemingly characterized by the lack all spermatogenic cells. However, tubules of some SCO testes contain small patches of active spermatogenesis and thus spermatogonial stem cells (SSCs). We hypothesized that these SSCs cannot replicate and seed spermatogenesis in barren areas of tubule because as of yet unrecognized deficits in Sertoli cell gene expression disable most stem cell niches. To test this hypothesis, we performed the first thorough comparison of the transcriptomes of human testes with complete spermatogenesis (n=4) with the transcriptomes of human testes with SCO syndrome (n=7) and we compared expression in normal and SCO testes of 204 transcripts that we defined by bioinformatic analyses as predominately expressed by human Sertoli cells. Seventy-one of these transcripts were expressed at similar levels in both sets of testes, while 75 and 58 were expressed at significantly higher and lower levels in the infertile organs. Some transcripts that were expressed at significantly higher levels are known to be essential for male fertility (e.g. Claudin 11, Nectin 2, KITL, WT1). However, some of the transcripts that were expressed at significantly lower levels encoded proteins required for the proper assembly of adherent and gap junctions at sites of contact with other cells, including SSCs. Others encode GDNF, FGF8 and BMP4, known regulators of mouse SSCs. Thus, specific deficits in gene expression by SCO Sertoli cells renders these cells incapable of organizing junctions at normal sites of cell-cell contact or stimulating SSCs with adequate levels of essential growth factors. We propose that these critical deficits are significant causes of some cases of human SCO syndrome.

Poster #49

IN VITRO EFFECTS OF THE ENDOCRINE DISRUPTORS GENISTEIN AND MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) ON INFLAMMATORY AND GERM CELL RESPONSES IN NEONATAL RAT TESTIS

Vanessa Brouard PhD and Martine Culty PhD
Department of Pharmacology and Pharmaceutical Sciences, University of Southern California
(Presented By: Vanessa Brouard)

Animal and epidemiological studies support the hypothesis that endocrine disruptor chemicals (EDCs) perinatal exposures affect the male reproductive system, leading to conditions comprised in the "Testicular dysgenesis syndrome". We previously found that fetal exposure to the plasticizer di-(2-ethylhexyl) phthalate (DEHP) and the phytoestrogen genistein (GEN), alone or in combination at a dose relevant to humans, altered testis morphology, transcriptome and function, in neonatal and adult rats. Innate immune cell markers were among the genes altered in EDC-exposed testes, suggesting the involvement of inflammatory processes. The goal of the present study was to examine the effects of GEN and MEHP, the main bioactive metabolite of DEHP, on inflammatory responses and germ cell profiles in neonatal testis, using an organo-culture system. Organo-cultures were performed with testes from postnatal day (PND) 2 and 3 rats, that were collected, cut into 4 to 6 small pieces, placed on filter papers and cultured in transwell-inserts, in the presence of medium, GEN and/or MEHP at 10-4 and 10-5 M, and/or 10 ng/ml of pro-inflammatory lipopolysaccharide (LPS). In PND2 testes, exposure to GEN and/or MEHP affected mainly the expression of germ cell specific genes. In PND3 testes, exposure to GEN and/or MEHP significantly decreased the expression of germ cell and stem cell markers, while GEN reduced AMH expression, suggesting altered Sertoli cell function. In inflammatory condition, the mRNAs of pro-inflammatory genes IL1b and Cxcl2 were increased by LPS, validating the use of organo-culture to study innate immune responses. Moreover, GEN+MEHP exposure reduced LPS-pro-inflammatory effects, while GEN significantly decrease IL1b and Cxcl2 levels. These results suggest that that GEN acts as an anti-inflammatory molecule by decreasing pro-inflammatory genes and cytokines expression in neonatal testis in basal conditions, while the GEN+MEHP mixture reduced LPS-pro-inflammatory responses in neonatal rat testis. Changes in gene expression showed that GEN and MEHP also targeted neonatal germ and Sertoli cells at a sensitive developmental window for the establishment of germline stem cells. These results suggest that some of the deleterious long term effects of GEN and MEHP may originate in perinatal effects on intra-testicular immune cells and perinatal germ cells.

Poster #50

LUTEOLIN MODULATES GENE EXPRESSION RELATED TO STEROIDOGENESIS, APOPTOSIS AND STRESS RESPONSE IN RAT LC540 TUMOR LEYDIG CELLS

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(Presented By: Mustapha Najih, BSc)

Androgen are mainly synthesized by testicular Leydig cells and play critical roles in testis development, normal masculinization, spermatogenesis, and male fertility. The rate-limiting step in testosterone biosynthesis involves the import of cholesterol inside mitochondria by the steroidogenic acute regulatory (STAR) protein. Cholesterol is then converted to pregnenolone by the steroidogenic enzyme CYP11A1, followed by a chemical transformation to testosterone using other steroidogenic enzymes. Interestingly, Star protein level declines during Leydig cell aging, resulting in defective mitochondrial cholesterol transfer and lower testosterone production. Previously, we have shown that 10 μM luteolin increased cAMP-dependent Star expression, leading to progesterone synthesis from mouse MA-10 Leydig cells. In this study, we examined whether polyphenolics having structural similarities to luteolin could promote steroidogenic and cancer related gene expressions within rat L540 tumor Leydig cells. In addition to Star activation, luteolin inhibited gene expression related to cholesterol biosynthesis and increased the expression of genes related to apoptosis, glutathione-S transferases and unfolded protein response. Thus, dietary luteolin may be effective in maintaining steroid production and inhibiting the proliferation of cancer cells within aging males.

POSTER ABSTRACTS

Poster #51

INVESTIGATION OF NOVEL MALE REPRODUCTIVE TRACT-SPECIFIC GENES AS CONTRACEPTIVE TARGETS

Kaori Nozawa PhD[1], Qian Zhang[2], Haruhiko Miyata[2], Zhifeng Yu[1], Darius Devlin[1], Ryan Matzuk[1], Masahito Ikawa[2] and Martin Matzuk[1]
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(Presented By: Kaori Nozawa, DVM, PhD)

Introduction: Development of a male contraceptive remains a long-held challenge in medicine. Male reproductive tract-specific proteins could be targets for non-hormonal contraception. Despite a large number of genes showing male reproductive organ-dominant expression and conservation in mammals, many of them are not essential for in vivo reproduction, as demonstrated by normal fertility in gene knockout (KO) mice. In this study, we selected three epididymis or testis-specific genes [epididymal protein 3B (Eddm3b), colipase-like 2 (Clpsl2), and serine-rich single-pass membrane protein 1 (Ssmem1)], and validated the in vivo significance of these genes.
Objectives: This study sought to generate KO mice for male reproductive tract-specific expressed genes and to evaluate the applicability of these proteins as contraceptive targets through phenotypic analysis. Methods: Using the CRISPR/Cas9 system, mutant mice with null alleles for each gene were generated. Sexually mature male mice were caged with females for several months and litter size was measured. KO mice showing male infertility were analyzed in more detail by evaluation of their testes and determination of morphology, counts, and motility of spermatozoa isolated from the caudal epididymis.
Results: Fecundity remained in KO males for Eddm3b and Clpsl2 (litter size: 7.6 ± 1.5 for Eddm3b KO, 9.1 ± 0.4 for Clpsl2 KO) whereas Ssmem1 KO males were sterile (litter size: 7.4 ± 1.8 for control, 0.0 for Ssmem1 KO). Ssmem1 KO mice showed abnormal sperm morphology (globozoospermia) and reduction in testis size and sperm motility.
Conclusions: We discovered that SSMEM1 is essential, but EDDM3B and CLPSL2 are dispensable for male reproductive function in mice although they are all expressed predominantly in the male reproductive tract. Ssmem1 KO males are sterile with defects in spermatogenesis. Further analysis of these mice has the potential for the development of a new contraceptive and the treatment for male infertility.
Acknowledgements: This research was supported by Eunice Kennedy Shriver National Institute of Child Health & Human Development grants P01 HD087157 and R01 HD088412, Bill & Melinda Gates Foundation grant OPP1160866, and a Japan Society for the Promotion of Science Overseas Research Fellowship.

Poster #52

GENE THERAPY FOR MALE INFERTILITY IN INDUCED-PLURIPOTENT STEM CELLS (IPSCS) DERIVED FROM TESTICULAR FIBROBLASTS OF AN INFERTILE MOUSE

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(Presented By: Chatchanan Doungkamchan, MD)

Azoospermia affects approximately 1% of men worldwide. Several single gene defects have been identified in men with idiopathic non-obstructive azoospermia, including SOHLH1, TEX11 and MCM8. We propose a gene therapy method to treat azoospermic men with single gene defects. We aim to provide a proof of principle in a Sohlh1-knockout (Sohlh1-KO) mouse model which has maturation arrest phenotype at undifferentiated spermatogonia stage. We hypothesized that iPSCs can be derived from testicular fibroblasts of a Sohlh1-KO mouse and gene targeting can be done in the iPSCs to restore Sohlh1 gene function. The resulting gene-targeted iPSCs will subsequently be differentiated into primordial gem cell-like cells (PGCLCs) from which sperm could be derived following transplantation. Our preliminary data suggested that iPSCs can be successfully derived from testicular fibroblasts of a Sohlh1 mouse by transiently expressing Yamanaka's factors (Klf4, Oct4, Sox2 and c-Myc) using Sendai virus vector. To assess pluripotency of the established Sohlh1-KO iPSC clone, we stained the iPSC clone at passage 11 with pluripotency markers and the clone was positive for alkaline phosphatase, Oct-4, Sox2, SSEA-1 and DPPA-2. The teratoma assay showed discernible tumor at two weeks after transplantation. Histology is underway. We then performed gene therapy in Sohlh1-KO iPSCs utilizing CRISPR/Cas9. We introduced sgRNAs targeting Rosa26 locus and donor DNA template with 1kb-long left and right homology arms and PGK-Puromycin-resistant gene-T2A-Sohlh1 cDNA-P2A-mCherry-polyA cassette by electroporation. Four days after puromycin selection, we found that the iPSCs electroporated with both sgRNAs/Cas9 protein and donor template are the only group that survived puromycin selection, not those that were electroporated with only sgRNAs/Cas9, or empty vectors with mCherry or no transfection control, showing proper expression of puromycin-resistant gene from the donor cassette. To confirm integration at Rosa26 locus, we performed PCR using primers outside and inside of the donor cassette and found positive integration, showing successful targeting of the Rosa26 locus in Sohlh1-KO iPSCs. Further studies include single colony expansion and sequencing to ensure correct and on-target integration. Subsequently the gene-corrected Sohlh1-KO iPSCs will be differentiated into PGCLCs and transplanted back to the Solhl1-KO hosts to make sperm. This study is supported by Magee-Womens Research Institute.

Poster #53

DIFFERENTIAL RA RESPONSIVENESS DIRECTS FORMATION OF THE FOUNDATIONAL SPERMATOGONIAL POPULATIONS AT THE INITIATION OF SPERMATOGENESIS IN THE MOUSE

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(Presented By: Christopher Geyer, PhD)

In the mammalian testis, the foundation of spermatogenesis is provided by spermatogonial stem cells (SSCs), a small subset of undifferentiated type A (Aundiff) spermatogonia. The progeny of SSCs either remain as stem cells (following a self-renewal division) or proliferate as undifferentiated progenitors prior to differentiating in response to retinoic acid (RA) and later entering meiosis. The mechanisms regulating the capacity of spermatogonia to respond to RA are undefined, and their identification would represent a key advance in our understanding of how spermatogonial fate is determined both at the beginning of spermatogenesis and throughout the male reproductive lifespan. Here, using the neonatal mouse testis as a model system, we report that the spermatogonial population is heterogeneous with respect to the capacity of individual cells to respond to RA. We found that differential responsiveness is provided by multiple mechanisms. The majority of type Aundiff progenitor spermatogonia are prevented from responding to RA by the catabolic activity of CYP26 enzymes. A smaller subset of spermatogonia that represents the putative SSC pool are RA-insensitive and protected from RA via a catabolism-independent mechanism. We propose a model by which spermatogonial fate is regulated by the ability of spermatogonia to ignore or respond to the differentiation signal provided by RA at the initiation of spermatogenesis.

POSTER ABSTRACTS

Poster #54

MUTATION IN FOXN1 GENE AFFECTS NORMAL TESTIS POST-NATAL DEVELOPMENT

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(Presented By: Gleide Fernandes Avelar, PhD)

A mutation in Foxn1 gene leads to the nude mice phenotype, characterized by the lack of thymus and lower serum levels of gonadotropins (LH and FSH) and testosterone. Previous work from our laboratory already showed several differences in adult nude mice testis in comparison to the wild type (WT). The most important results regard Leydig cells (LC): nude have a higher population of those cells, with a smaller volume. Furthermore, the expression of several steroidogenic enzymes was higher in nude, corresponding to a compensatory effect, since they have smaller levels of testosterone. The expression of the transcription factor Foxn1 found in LC explains the regulation of the steroidogenic enzymes production. In order to better understand if these peculiar reproductive aspects occur in nude mice due to alterations during testis development, we conducted an investigation of the testicular function in nude BALB/c male mice in several ages of post natal development (Pnd10, 15 and 20). Besides evaluating biometric and histomorphometric data, we also measured testosterone serum levels and, by immunohistochemistry, we investigated Foxn1 expression. Interestingly, we found that, since body and testis weights were smaller in nude than in the WT in several ages, a similar gonadosomatic index (testis mass/body weight) was found for both strains at all ages investigated. Even though differences were absent for the Sertoli cell parameters investigated, total length of seminiferous tubules per gram of testis was smaller in WT at Pnd10 and 15. Most importantly, we found that, already at Pnd10, nude mice have smaller LC nuclear volume and, at Pnd15 and 20, the individual cell volume was also smaller, pattern that maintains until adulthood. On the other hand, the LC population did not change between WT and nude in any of the different ages. This implies that, probably, the higher number of LC found in adults is determined only in adult age. As a way to analyse LC function, the testosterone serum level was measured, but no differences were found at any of the ages investigated. Another important result is the differential expression of Foxn1 along the development: the number of interstitial cells stained and the intensity of the expression grows as the mice ages, coinciding with the switching from fetal to adult LC population. This indicates that, apparently, the fetal LC does not express this transcription factor, thus, Foxn1 seems to be a marker of the adult population.

Poster #55

FUNCTIONAL ROLE OF THE COPPER TRANSPORTER 1 PROTEIN IN SPERMATOGENESIS AND IN CISPLATIN-INDUCED TESTICULAR INJURY

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(Presented By: Rashin Ghaffari, BS)

Cisplatin (cDDP) is a highly effective chemotherapeutic drug. However, treatment with cDDP contributes to many adverse side effects including prolonged azoospermia in male patients. Both acute and sub-chronic exposure to cDDP induces testicular germ cell (GC) loss and prolonged azoospermia, respectively, in animal models. Although it has been well documented that cDDP exposure induces male reproductive toxicity and disruption to spermatogenesis, the mechanism (s) that account for the prolonged infertility as a result of cDDP treatment is not understood. The high affinity membrane copper transporter 1 (CTR1; SCL31A1) protein, which functions as a copper (Cu) importer in mammalian cells, has been shown to be highly associated with cDDP sensitivity and accumulation in mammalian cells. Our preliminary evaluation on mice testis indicates that CTR1 is primarily expressed in primary spermatocytes and SCs. To examine the role of CTR1 in cDDP-induced testicular injury, we have developed two independent mouse models, with the conditional knockout of Ctr1 in either SCs (SC-KO; Amh-Cre, Ctr1fl/Δ) or GCs (GC-KO; Ddx4-Cre, Ctr1fl/Δ). Interestingly, GC-KOs exhibit a severe reduction in testis weight (~83% by PND 41) with complete depletion of post-meiotic GCs. On the other hand, SC-KO mice were fertile and had indistinguishable testis weight and histology from their wild-type (WT; Ctr1fl/fl) littermates. The SC-KO mice were further challenged with an acute dose of cDDP, where the SC-KO and WT mice were either exposed to a single high dose of 5 mg/kg of cDDP or equivalent volume of saline for 48 hours. We found that while cDDP treated WT mice had twofold increase in GC death compared to saline treated WT mice, cDDP treated SC-KO mice exhibited only half fold increase in GC death compared to saline treated SC-KO mice. Moreover, platinum levels in cDDP treated SC-KO mice testis were significantly lower than in WT mice testis. Taken together, these observations reveal for the first time 1) the essential role of CTR1 in GCs, but not in SCs, for functional spermatogenesis and, 2) the functional significance of CTR1 expressed by SCs on mediating cDDP-induced GC loss. Future investigations will utilize SC-KO as a mouse model to study the contribution of the environmental niche provided by the SC on spermatogenesis followed by clinically relevant multi-dose cDDP treatment, and the GC-KO mice will be utilized to explore the importance of CTR1 and/or Cu on spermatogenesis.

Poster #56

REGULATION OF BLOOD-TESTIS BARRIER PROTEINS THROUGH NOTCH NON-CANONICAL PATHWAYS

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(Presented By: Parag Parekh, PhD)

Sertoli-Sertoli cells junctions separate the seminiferous epithelium into basal and adluminal compartments, forming the blood-testis-barrier (BTB) in the adult testis. Junctions undergo disassembly and reassembly as germ cells migrate toward the epithelium's lumen during maturation. Key events of spermatogenesis take place during stage VII-VIII of the seminiferous epithelium cycle, including differentiation of Aundiff into A1 spermatogonia, meiotic entry of spermatocytes, initiation of spermatid elongation, as well as major BTB remodeling. The mechanism(s) coordinating these events are not completely understood. We previously demonstrated, using Sertoli cell-specific NOTCH signaling gain and lack of function, that the NOTCH canonical pathway downregulates Gdnf and Cyp26b1 through activation of RBPJ and expression of the transcriptional repressors HES1/HEY1. Microarray, RNA-Seq and qPCR data indicated that NOTCH activity peaks at stage VII-VIII of the seminiferous epithelium cycle, and might also upregulate the expression of some BTB genes/proteins at these stages. We hypothesized that activated NOTCH may coordinate the regulation of these molecules through a non-canonical pathway that does not involve HES1/HEY1. Promoter analysis using the Eukaryotic Promoter Database showed multiple putative RBPJ binding sites at the proximal promoter of Gja1, Cdh2, Nectin2, Cldn11 and Tjp2, possibly directly regulating their expression. We isolated adult Sertoli cells from Amh-Cre; RosaRFP/RFP transgenic mice and cultured these cells for 48h with the NOTCH ligand JAG1, the NOTCH inhibitor DAPT and a combination of JAG1 and DAPT. qPCR and Western blot analysis of these Sertoli cells revealed upregulated expression of these genes/proteins. Sertoli cells from Amh-Cre; RBPJfl/fl; RosaYFP/YFP mice (Rbpj-KO Sertoli cells) showed significant downregulation of these genes in comparison to controls. In addition, the histology of RBPJ knockout mice testes showed significant lengthening of stage VIII of the epithelium

cycle supporting alteration of BTB reassembly. In conclusion, RBPJ might be a multifunctional transcription factor able to simultaneously up- or downregulate BTB proteins and other Sertoli cell factors, playing an important role in the coordination of the events of stages VII-VIII of the seminiferous epithelium. Funded by NIH-HD081244

Poster #57

LOSS OF FAM170A CAUSES SUBFERTILITY AND DEFECTIVE SPERM MOTILITY IN MICE

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(Presented By: Darius J. Devlin, BS)

Introduction: Family with sequence similarity 170 members A and B (FAM170A and FAM170B) are two testis-specific, acrosome-localized paralogous proteins conserved throughout mammals. While in vitro experiments in previous literature suggested FAM170B plays a role in mouse sperm acrosome reaction, the role of FAM170A in the testis has not been explored.
Objectives: This work sought to generate single and double knockout (KO) models for the Fam170 genes and evaluate the consequences of gene loss on male fertility. Methods: Using CRISPR/Cas9, null alleles for each gene were generated and homozygous null male mice were mated to wildtype (WT) females for six months to assess fertility. Epididymal sperm from adult males was analyzed by Computer Assisted Sperm Analysis (CASA) software to assess sperm motility and kinetics. Light microscopy images of extracted epididymal sperm were used to assess mature sperm gross morphology.
Results: Mating experiments revealed significantly reduced average litter sizes from Fam170a KO males compared to heterozygous control males. Though both Fam170a KO and control males had normal testis weights, CASA experiments revealed a significant reduction in Fam170a KO sperm count. Although overall percent motility was similar to control males, KO males had dramatically reduced progressive motility. In addition, light microscopy of Fam170a KO sperm revealed abnormal sperm head morphology and a bent neck. In contrast, Fam170b KO males produced similar average litter sizes when compared to their controls, sperm counts were only slightly reduced, and sperm morphology appeared normal.
Conclusions: FAM170A has an important role in male fertility, as loss of the protein leads to subfertility; however, it is unclear whether the FAM170B paralog gives partial rescue of fertility in these KO males. We have begun assessing the fertility of Fam170a/Fam170b double KO males to determine if there is any redundancy. This work will further our molecular understanding of sperm function and could help improve male infertility diagnoses. Acknowledgements: Supported by Eunice Kennedy Shriver National Institute of Child Health & Human Development grants P01 HD087157 and R01 HD088412, the Bill & Melinda Gates Foundation grant OPP1160866, and training fellowships from the Interdepartmental Program in Translational Biology and Molecular Medicine grant T32 GM088129 and the Training Interdisciplinary Pharmacology Scientists (TIPS) Program grant T32 GM120011.

Poster #58

TRANSCRIPTOMIC ANALYSIS OF OVEREXPRESSED SOX8 IN TM4 SERTOLI CELLS WITH EMPHASIS ON CELL-TO-CELL INTERACTIONS

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(Presented By: Luc J. Martin, PhD)

Sertoli cells are located in seminiferous tubules within the testis. They are the first testicular cells to differentiate during male sex determination. In the adult, Sertoli cells provide nutrients to germ cells, control factors for spermatogenesis and protection by establishing the blood-testis barrier (BTB). This BTB is composed of tight junctions, basal ectoplasmic specializations, adherent junctions and gap junctions. The transcription factor SOX8 is necessary for the maintenance of spermatogenesis during adult life and is highly expressed in Sertoli cells. However, its targets genes in adult Sertoli cells remain to be characterized. Hence, we compared the transcriptomes of TM4 Sertoli cells overexpressing or not SOX8 by using RNA-Seq followed by pathways and networks analyses. We found that upregulated genes in response to SOX8 overexpression were enriched for Sertoli cell development and differentiation. However, downregulated genes were enriched for cell-to-cell adhesion, tight junction interactions, gap junctions' assembly, as well as extracellular matrix binding. Hence, our results confirm that SOX8 is an important mediator of Sertoli cell maturation and of gene expression related to regulation of blood-testis barrier assembly. In addition, TM4 cells can be considered as a useful model to better define the regulatory mechanisms of SOX8 on gene transcription in Sertoli cells.

Poster #59

TESTIS SPECIFIC MAGE GENES EVOLVED TO PROTECT MAMMALIAN SPERMATOGENESIS UNDER STRESS

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(Presented By: Klementina Fon Tacer, DVM, PhD)

The basic processes of spermatogenesis are surprisingly similar in even very distinct species, and the genes responsible are highly conserved. However, the majority of reproductive genes evolve faster than their non-reproductive counterparts and drive lineage-specific evolution. One of the evolutionarily most intriguing classes of genes is testis-specific genes that are expressed exclusively in spermatogenesis. Their function, in particular in mammals, is still poorly understood. Here, we describe a family of genes called melanoma antigens (MAGEs) that evolved in eutherian mammals and are normally restricted to expression in the testis but are often aberrantly activated in cancer. Depletion of Mage-a genes disrupted spermatogonial stem cell maintenance in culture and impaired repopulation efficiency in vivo. Exposure of Mage-a knockout mice to genotoxic stress or long-term starvation that mimics famine in nature causes defects in spermatogenesis, impaired recovery after stress and reduced fertility. Finally, human MAGE-As are activated in many cancers where they confer resistance to metabolic stress and promote cell growth. These results suggest that mammalian-specific MAGE genes evolved to protect the male germline against diverse stressors, ensure reproductive success under non-optimal conditions, and are hijacked by cancer cells.

POSTER ABSTRACTS

Poster #60

DHT ADMINISTRATION ALTERS COLLAGEN ORGANIZATION IN THE CRANIAL SUSPENSORY LIGAMENTS IN GLI3XTJ MUTANT MICE

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(Presented By: Anbarasi Kothandapani, PhD)

Introduction: Cryptorchidism is the most frequent congenital birth defect in male children and can occur as an isolated disorder or associated with other congenital anomalies such as Greig cephalopolysyndactyly syndrome (GCPS). GCPS is an autosomal dominant disorder that affects craniofacial and limb development and many of the affected males have genital abnormalities including cryptorchidism and/or hypospadias. GCPS is caused by loss of function mutations in the Hedgehog pathway mediator, GLI3, and presents as a wide spectrum of phenotypes with variable severity.
Hypothesis: While it is established that Desert Hedgehog (DHH) signaling is essential for the onset of testicular androgen synthesis for normal male sex differentiation, elimination of individual downstream mediators, GLI1 or GLI2, had no effect. Therefore, we hypothesize that GLI3 is required for DHH-mediated androgen synthesis and hence, normal testicular descent.
Methods: To test our hypothesis, we used the Extra-toes (Gli3XtJ) mouse, an established animal model for GCPS. The Gli3XtJ mutation is a 51.5kb deletion in the zinc finger region of the Gli3 locus that results in a null phenotype. We examined testosterone production and testicular descent as a function of fetal Leydig cells.
Results: Our results showed diminished fetal Leydig cell number in Gli3XtJ mutants without any changes in testicular morphology. Transcript levels of Insl3 was unchanged, but steroidogenic genes were significantly lower resulting in less testosterone production in mutant testes. We observed delayed testis descent in all Gli3XtJ mutants due to failed disintegration of the cranial suspensory ligament (CSL). At the site of insertion, the mutant CSL was shorter and thicker, but androgen receptor expression was unchanged. To test whether low testosterone caused the delayed descent, we treated pregnant dams with DHT or vehicle control. Although DHT treatment did not hasten descent, the collagen content of the CSL was affected. Compared to vehicle treated mutants, CSLs in DHT treated mutants showed lesser collagen density with reduced fiber length and angle.
Conclusions: The loss of Gli3 caused a reduction in Leydig cell numbers and androgen production leading to failed CSL regression and delayed testicular descent. DHT administration was not sufficient to rescue the delayed testicular descent; however, it was sufficient to reorganize collagen to facilitate CSL degeneration. Funding: NIH HD090600

Poster #61

IN VITRO IMPACT OF THE ENDOCRINE DISRUPTORS GENISTEIN AND MONO-(2-ETHYLHEXYL) PHTHALATE ON THE EICOSANOID PATHWAY IN SPERMATOGONIAL STEM CELLS

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(Presented By: Amy Tran, MS)

The eicosanoid biosynthesis pathway was shown to play a critical role in fetal testis and germ cell development in rat and human, based on studies using the cyclooxygenase (COX) inhibitors NSAIDs and acetaminophen. Indeed, COX inhibition was found to reduce fetal testis levels of prostaglandins D2 and/or E2, in association with cryptorchidism, altered Leydig and Sertoli cell function and decreased fetal germ cell markers. Perinatal exposure to endocrine disruptor chemicals (EDCs) has been shown to be disruptive to normal spermatogenesis, and studies, including ours, have found that mixtures of EDCs at environmentally relevant doses can induce alterations in the testis, leading to male reproductive pathologies. The goal of this in vitro study was to determine whether exposure of spermatogonial stem cells (SSCs) to the EDC genistein (GEN) and the bioactive metabolite of DEHP, Mono-(2-ethylhexyl) phthalate (MEHP), either alone or in combination, could disrupt the eicosanoid biosynthesis pathway and interfere with spermatogonial functions. The mouse C18-4 spermatogonial cell line (model for undifferentiated spermatogonia including SSCs) was treated with GEN and/or MEHP at 10−5 or 10−4M, for 24 and 48 hours. 24-hour treatment of GEN and GEN+MEHP at 10-5M and 10-4M significantly reduced the gene expression of PLA2, the enzyme responsible for producing arachidonic acid, and the substrate of COX enzymes. This effect was sustained after 48 hours with GEN and GEN+MEHP 10-4M treatments. While COX-2 gene expression was significantly increased by GEN and GEN+MEHP 10-4M treatments, COX-1 gene expression was significantly decreased at both 24- and 48-hour time points. Prostaglandin D synthase gene expression significantly increased with GEN and GEN+MEHP 10-4M treatment, whereas the same treatments lowered Prostaglandins I and E synthase expression. Simultaneously, the gene expression of the SSC marker ID4 was significantly decreased, while several markers of undifferentiated spermatogonia increased, suggesting a GEN-driven switch from SSCs to transitional spermatogonial progenitors. These data suggest that exposure to environmentally relevant doses of GEN but not MEHP alone can disrupt the eicosanoid pathway in different manners, and potentially interfere with the mechanisms regulating self-renewal processes in spermatogonia.

Poster #62

SINGLE-CELL RNA SEQUENCING REVEALS NOVEL MARKERS OF STEM/PROGENITOR SPERMATOGONIA IN HIGHER PRIMATES

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(Presented By: Sarah Munyoki, BA)

In the adult male testis, spermatogonial stem cells (SSCs) are essential for continuous spermatogenesis to maintain fertility throughout life. SSCs exquisitely balance self-renewal and differentiation through molecular mechanisms that are still poorly understood, especially in higher primates. SSC transplantation as a therapy for male infertility, is well established in rodents and may have application in the human clinic. However, while many features of testicular biology are conserved from rodents to higher primates, there is divergence in stem cell phenotype and spermatogenetic lineage development. Determining the unique features of higher primate SSCs will facilitate the translation of SSC based therapies to the human fertility clinic. We employed Drop-Seq for high throughput, unbiased, single-cell RNA-sequencing of healthy adult higher primates (human and rhesus macaque) testicular tissue, generating ~33,800 single cell transcriptomes. Dimensionality reduction and unsupervised clustering methods partitioned the cells into transcriptionally distinct populations, representing all known and potentially novel cell types of higher primate testes. Further analysis of our data has identified novel genes GPX1, MORC1, GPC4 and GPC3 as potential markers of human and monkey stem/progenitor spermatogonia. These genes are known to have diverse cellular functions and are involved in various signaling pathways such as WNT, Hedgehog, FGF, BMP, MAP2K/AKT and degradation of reactive oxygen species that may be important in regulating higher primate SSC function. Our single cell data may reveal novel mechanisms regulating higher primate SSCs that can be exploited for sorting, enhancing survival and expansion in culture or other applications that improve the fundamental knowledge about SSCs in higher primates, and may enable applications in the male infertility clinic.

POSTER ABSTRACTS

Poster #63

HORMONOTHERAPY AND RADIOTHERAPY ON SPERMATOGENESIS IN HUMAN: MATURATION ARREST AND SPERMATOGONIAL PROLIFERATIVE ACTIVITY

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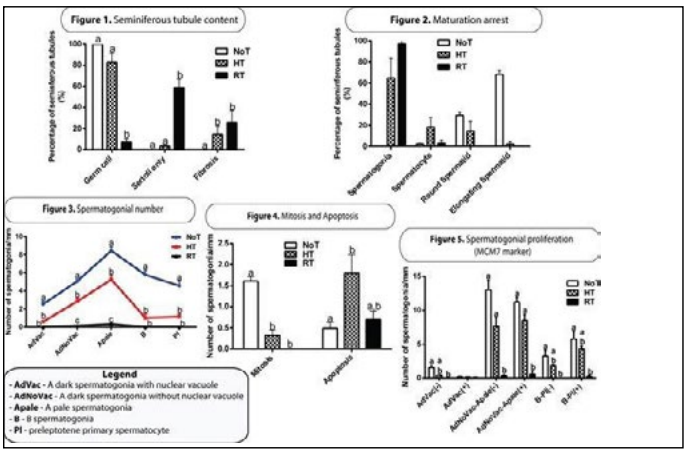
(Presented By: Helio Chiarini-Garcia, PhD)

Numerous studies have shown in rodents the effects of hormonal (HT) and radiotherapy (RT) treatments on the spermatogenic process. However, in humans there are few studies in this direction, mainly those that evaluate morphofunctional parameters of spermatogenesis and the stem spermatogonial behavior. Therefore, the present study aimed to evaluate the testis from patients diagnosed with prostate cancer and underwent HT or RT, compared to others who also presented prostate cancer, but were not submitted to any treatment (NoT). Testis fragments of 15 men (n=5 per group) were fixed in glutaraldehyde for high-resolution light microscopy, as well as fixed in paraformaldehyde for immunohistochemistry (MCM7 proliferative marker). While in NoT patients all seminiferous tubules (ST) presented germ cells (GC), in RT the majority showed Sertoli Only and fibrotic ST (Fig. 1). In HT testis, around 80% of ST presented GC while few of them were fibrotic. Evaluating the maturation arrest (Fig. 2), the majority of ST after HT and RT was arrested in spermatogonia. Again, in RT testis, it was hardly seen ST in which GC reached the meiotic step. Regarding spermatogonial numbers (Fig. 3), while they were low in HT ($p<0.05$), they were drastically reduced in RT testis ($p<0.05$). Interestingly, AdVac spermatogonia, which have been proposed as the stem one, were not observed in RT. In fact, the number of spermatogonial mitosis (Fig. 4) was reduced ($p<0.05$) after HT and RT. Evaluating spermatogonial apoptosis, it was seen that its number was significantly increased after HT, while the spermatogonial mitotic activity through the MCM7 marker (Fig. 5) was drastically decreased after RT in all generations (AdVac, AdNoVac, Apale and B). Following HT, the proliferative activity of all spermatogonial subtypes was maintained. We concluded that RT shows more severe damage to spermatogenesis and spermatogonial mitotic activity than HT. On the other hand, as the HT maintains the spermatogonial proliferative capacity, this method may be more suitable for prostate cancer treatment when spermatogenesis recovery is expected. Support Grant: CNPq, CAPES, FAPEMIG.



Poster #64

EFFECTS OF MATRIX-BOUND NANOVESICLES IN HUMAN SPERMATOGONIAL STEM CELL CULTURE

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(Presented By: Kien T. Tran, Bachelor)

Spermatogonial stem cells (SSCs) are at foundation of spermatogenesis and essential for male fertility. Cancer patients who undergo chemotherapy and radiation treatments encounter a significant risk of stem cell pool depletion which can lead to permanent infertility. Since prepubertal boys are not yet producing sperm, they can only preserve testicular biopsies. Prepubertal testicular tissues house SSCs which can be used in tissue-based or cell-based therapies to produce sperm in the future. SSC transplantation is a promising technology used to restore fertility. However, the number of human SSCs (hSSCs) recovered from a small testis biopsy from a young patient may be limited. Therefore, establishing a culture method to expand hSSCs in vitro is a crucial step toward cell-based therapy. Extracellular matrixes (ECMs) have been used as bioscaffolds in regenerative medicine to support survival and growth various cell types, in vivo and ex vivo. We recently reported that human testis ECM substrate in hSSC culture yielded a significantly higher number of undifferentiated spermatogonia during a 14-day culture period compared to STO feeder cells. The current study will specifically test the bioactivity of human testis ECM-bound nanovesicles (MBVs), which are known to carry microRNAs, cytokines, chemokines, and other proteins that that could impact survival, proliferation, and differentiation of hSSCs in culture. We tested MBVs derived from human testis, porcine testis, porcine urinary bladder and porcine small intestinal submucosa. We evaluated cultures using the high-throughput flow cytometry method that can simultaneously analyze multiple developmental stages of the cultured cells. Our preliminary data showed that MBVs are internalized by hSSCs. We did not observe differences among the MBV culture conditions in our initial 14 day culture experiments, but dosing studies are currently underway. This work was supported by the Eunice Kennedy Shriver National Institute for Child Health and Human Development grant HD092084 and the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health grant 5T32EB001026-15.

Poster #65

DAZL IS A MASTER TRANSLATIONAL REGULATOR OF MURINE SPERMATOGENESIS

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(Presented By: Eugene Xu, PhD)

Expression of DAZ-like (DAZL) is a hallmark of vertebrate germ cells and essential for embryonic germ cell development and differentiation, yet gametogenic function of DAZL has not been fully characterized with most of its in vivo direct targets unknown. We showed that postnatal stage-specific deletion of Dazl in mouse germ cells did not affect female fertility, but caused complete male sterility with gradual loss of spermatogonial stem cells (SSCs), meiotic arrest and spermatid arrest respectively. Using the genome-wide HITS-CLIP and mass spectrometry approach, we found that DAZL bound to a large number of testicular mRNA transcripts (at least 3008) at 3' untranslated region (3' UTR) and interacted with translation proteins including PABP. In the absence of DAZL, polysome-associated target transcripts, but not their total transcripts were significantly decreased, resulting in drastic reduction of an array of spermatogenic proteins and thus developmental arrest. Thus, DAZL is a master translational regulator essential for spermatogenesis.

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